PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

24743

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) **International Patent Classification** 5 :
C12N 15/12, 15/67, 9/90, 15/10, 5/10, C12P 21/08, C07K 7/06, C12Q 1/68, G01N 33/68, A61K 37/02

A1

(11) **International Publication Number:** **WO 94/20618**

(43) **International Publication Date:** 15 September 1994 (15.09.94)

(21) **International Application Number:** PCT/US94/02519

(22) **International Filing Date:** 8 March 1994 (08.03.94)

(30) **Priority Data:**
08/033,086 9 March 1993 (09.03.93) US

(71) **Applicant:** BOARD OF TRUSTEES OF THE UNIVERSITY OF ILLINOIS [US/US]; 352 Henry Administration Building, 506 South Wright Street, Urbana, IL 61801 (US).

(72) **Inventors:** RONINSON, Igor, B.; 2731 Lincoln Lane, Wilmette, IL 60091 (US). GUDKOV, Andrei; Apartment 1S, 5819 South Blackstone, Chicago, IL 60637 (US).

(74) **Agent:** NOONAN, Kevin, E.; Allegretti & Witcoff, Ltd., Ten South Wacker Drive, Chicago, IL 60606 (US).

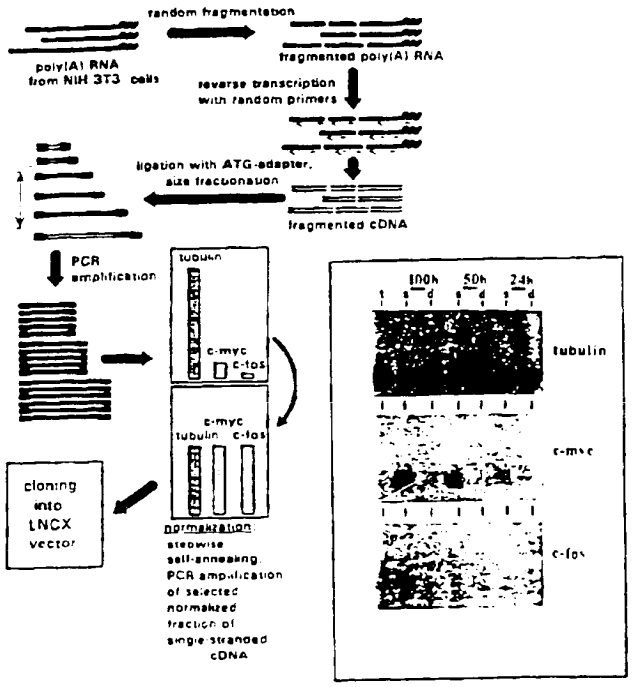
(81) **Designated States:** AU, CA, JP, KR, NO, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) **Title:** GENETIC SUPPRESSOR ELEMENTS ASSOCIATED WITH SENSITIVITY TO CHEMOTHERAPEUTIC DRUGS

(57) **Abstract**

The invention provides genetic suppressor elements that confer upon a cell resistance to one or more chemotherapeutic drug, methods for identifying and obtaining such elements, and methods of using such elements. The invention also provides cloned genes associated with sensitivity to chemotherapeutic drugs.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**GB**	United Kingdom	**MR**	Mauritania
AU	Australia	**GE**	Georgia	**MW**	Malawi
BB	Barbados	**GN**	Guinea	**NE**	Niger
BE	Belgium	**GR**	Greece	**NL**	Netherlands
BF	Burkina Faso	**HU**	Hungary	**NO**	Norway
BG	Bulgaria	**IE**	Ireland	**NZ**	New Zealand
BJ	Benin	**IT**	Italy	**PL**	Poland
BR	Brazil	**JP**	Japan	**PT**	Portugal
BY	Belarus	**KE**	Kenya	**RO**	Romania
CA	Canada	**KG**	Kyrgyzstan	**RU**	Russian Federation
CF	Central African Republic	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CG	Congo			**SE**	Sweden
CH	Switzerland	**KR**	Republic of Korea	**SI**	Slovenia
CI	Côte d'Ivoire	**KZ**	Kazakhstan	**SK**	Slovakia
CM	Cameroon	**LI**	Liechtenstein	**SN**	Senegal
CN	China	**LK**	Sri Lanka	**TD**	Chad
CS	Czechoslovakia	**LU**	Luxembourg	**TG**	Togo
CZ	Czech Republic	**LV**	Latvia	**TJ**	Tajikistan
DE	Germany	**MC**	Monaco	**TT**	Trinidad and Tobago
DK	Denmark	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**US**	United States of America
FI	Finland	**ML**	Mali	**UZ**	Uzbekistan
FR	France	**MN**	Mongolia	**VN**	Viet Nam
GA	Gabon				

GENETIC SUPPRESSOR ELEMENTS ASSOCIATED WITH SENSITIVITY TO CHEMOTHERAPEUTIC DRUGS.

BACKGROUND OF THE INVENTION

1. Field Of The Invention

The invention relates to genetic factor associated with sensitivity to chemotherapeutic drugs. More particularly, the invention relates to methods for identifying such factors as well as to uses for such factors.

2. Summary Of The Related Art

A broad variety of chemotherapeutic agents are used in the treatment of human cancer. For example the textbook *CANCER: Principles & Practice of Oncology*, 2d Edition, (De Vita *et al.*, Eds.), J.B. Lippincott Company, Philadelphia, PA (1985) discloses a major antineoplastic agents the plant alkaloids vincristine, vinblastine, vindesine, and VM-26; the antibiotics actinomycin-D, doxorubicin, daunorubicin, mithramycin, mitomycin C and bleomycin; the antimetabolites methotrexate, 5-fluorouracil, 5-fluorodeoxyuridine, 6-mercaptopurine, 6-thioguanine, cytosine arabinoside, 5-aza-cytidine and hydroxyurea; the alkylating agents cyclophosphamide, melphalan, busulfan, CCNU, MeCCNU, BCNU, streptozotocin, chlorambucil, bis-diaminedichloro-platinum, azetidinylbenzoquinone; and the miscellaneous agents dacarbazine, mAMSA and mitoxantrone.

These and other chemotherapeutic agents such as etoposide and amsacrine have proven to be very useful in the treatment of cancer. Unfortunately, some tumor cells become resistant to specific chemotherapeutic agents, in some instances even to multiple chemotherapeutic agents. such drug resistance or multiple drug resistance can theoretically arise from either the presence of genetic factors that confer resistance to the drugs, or from the absence of genetic factors that confer sensitivity to the drugs. The former type of factors have been identified, and include the multiple drug resistance gene *mdr-1* (see Chen *et al.*, 1986, *Cell* 47: 381-389). However, the latter type of factor remains largely unknown, perhaps in part because such absence of factors would tend to be a recessive trait.

Identification of genes associated with sensitivity to chemotherapeutic agents is desirable, because the discovery of such genes can lead to both diagnostic and therapeutic approaches for cancer cells and for drug resistant cancer cells, as well as to improvements in gene therapy and rational drug design. Recently, some developments have been made in the difficult area of isolating recessive genetic elements, including one involved in cytotoxic drug sensitivity. Roninson et al., U.S. Patent No. 5,217,889, issued June 8, 1993, teaches a generalized method for obtaining genetic suppressor elements (GSEs), which are dominant negative factors that confer the recessive-type phenotype for the gene to which the particular GSE corresponds. (*See also* Holzmayer *et al.*, 1992, *Nucleic Acids Res.* 20: 711-717). Gudkov *et al.* (1993, *Proc. Natl. Acad. Sci. USA* 90: 3231-3235) teaches isolation of GSEs inducing resistance to topoisomerase II-interactive drugs from topoisomerase II cDNA. However, there remains a need for identifying yet unknown genes or genetic elements associated with sensitivity to chemotherapeutic agents, a task made more difficult by the unavailability of a cloned gene as starting material for preparing GSEs. Preferably, such genes or genetic elements will be involved in a common pathway that is implicated in sensitivity to more than one chemotherapeutic agent. Most preferably, such genes or genetic elements will be identified by direct selection of GSEs causing loss of the drug sensitivity phenotype.

BRIEF SUMMARY OF THE INVENTION

The invention provides genetic suppressor elements (GSEs) that confer upon cells resistance to chemotherapeutic drugs. These GSEs are random fragments derived from genes associated with sensitivity to chemotherapeutic drugs, although the nature of such genes can be quite surprising.

In a first aspect, the invention provides a method for identifying GSEs that confer resistance to any chemotherapeutic drug for which resistance is possible. this method utilizes chemotherapeutic drug selection of cells that harbor clones from a random fragment expression library derived from total

cDNA and subsequent rescue of library inserts from drug-resistant cells. In a second aspect, the invention provides a method for identifying and cloning genes that are associated with sensitivity to chemotherapeutic drugs, including genes that have not been previously discovered. This method comprises the steps of screening a full length cDNA library with a GSE that confers upon cells resistance to chemotherapeutic (or an oligonucleotide or polynucleotide constituting a portion of such a GSE) and determining the nucleotide sequence of the cDNA insert of any positive clones obtained. In a third aspect, the invention provides a method for obtaining GSEs having optimized suppressor activity for a gene associated with sensitivity to a chemotherapeutic drug. This method utilizes chemotherapeutic drug selection of cells that harbor clones from a random fragment expression library derived from DNA of a gene associated with sensitivity to the same chemotherapeutic drug, and subsequent rescue of the library inserts from drug resistant cells. In a fourth aspect, the invention provides synthetic peptides and oligonucleotides that confer upon cells resistance to chemotherapeutic drugs. These synthetic peptides and oligonucleotides are designed based upon the sequence of a drug-resistance conferring GSEs according to the invention.

In a fifth aspect, the invention provides a diagnostic assay for tumor cells that are resistant to one or more chemotherapeutic drug due to the absence of expression or underexpression of a particular gene. This diagnostic assay comprises quantitating the level of expression of the particular gene product by a particular tumor cell sample to be tested. In sixth aspect, the invention provides dominant selectable markers that are useful in gene co-transfer studies. These dominant selectable markers are drug resistance-conferring GSEs according to the invention operably linked to appropriate transcriptional control elements. In a seventh aspect, the invention provides *in vivo* selectable markers that are useful both for gene therapy and for enhanced chemotherapy for cancer. Such *in vivo* selectable markers are transferred into blood progenitor cells, which are then used to repopulate the patient's blood exclusively with cells that contain a co-transferred therapeutic

gene, or for chemotherapy, just the chemotherapeutic drug resistance conferring GSE. In an eighth aspect, the invention provides a starting point for the rational design of pharmaceutical products that are useful against tumor cells that are resistant to chemotherapeutic drugs. By examining the structure, function, localization and pattern of expression of genes associated with sensitivity to chemotherapeutic drugs, strategies can be developed for creating pharmaceutical products that will overcome drug resistance in tumor cells in which such genes are either not expressed or underexpressed.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the nucleotide sequences of twelve GSEs that confer etoposide resistance upon cells and that were derived from topoisomerase II DNA, using a single gene random fragment expression library, as described in Example 1. The GSEs shown are (A) clone 2V [SEQ. ID. No. 1], (B) clone Σ11 [SEQ. ID. No. 2] (c) clone 6 [SEQ. ID. NO. 3, (D) clone 5 [SEQ. ID. NO. 4], (E) clone Σ28 [SEQ. ID. NO. 5], (F) clone Σ2 [SEQ. ID. No. 6], (G) clone Σ20 [SEQ. ID. No. 7], (H) clone 39 [SEQ. ID. NO. 8], (I) clone 12S [SEQ. ID. NO. 9], (J) clone Σ8 [SEQ. ID. NO. 10], (K) clone ΣVPs2 [SEQ. ID. NO. 11), and (L) clone ΣVM [SEQ. ID. NO. 12].

Figure 2 shows a scheme for construction of RFEL from NIH 3T3 cDNA. Panel A shows the overall construction scheme. Panel B shows normalization of the cDNA fragments. In this panel, t represents total unfractionated cDNA, s and d represent the single-stranded and double-stranded fractions separated by hydroxyapatite, time points indicate the period of reannealing, and tubulin, c-myc, and c-fos indicate the probes used in Southern hybridization with the total, single-stranded and double-stranded fractions.

Figure 3 shows the structure of the LNCX vector and the adaptor used in cDNA cloning. The nucleotide sequences are shown for the ATG-sense [SEQ. ID. NO. 13] and ATG-antisense [SEQ. ID. NO. 14] strands of the adaptor.

Figure 4 shows the overall scheme for selecting cell lines containing chemotherapeutic drug resistance-conferring GSEs and rescuing the GSEs from these cells.

Figure 5 shows etoposide resistance conferred by preselected virus (Panel A) and PCR analysis of the selected and unselected populations (Panel B).

Figure 6 shows a scheme for recloning individual PCR-amplified fragments from etoposide resistant selected cells into the LNCX vector, as described in Example 4.

Figure 7 demonstrates resistance to 350 ng/ml etoposide, conferred upon the cells by the GSEs VPA (Panel A) and VP9-11 (Panel B).

Figure 8 shows resistance to various concentrations of etoposide, conferred upon the cells by the GSE anti-*khcs* under an IPTG-inducible promoter (Panel A) and the scheme for this selection (Panel B).

Figure 9 shows the nucleotide sequence of the GSE anti-*khcs* [SEQ. ID. NO. 15].

Figure 10 shows the nucleotide sequence of the GSE VPA [SEQ. ID. NO. 16].

Figure 11 shows the nucleotide sequence of the GSE VP9-11 [SEQ. ID. NO. 17].

Figure 12 shows the nucleotide sequence of the most of the coding region of the mouse *khcs* cDNA [SEQ. ID. NO. 18].

Figure 13 shows the dot matrix alignments of *khcs* protein sequence deduced from the nucleotide sequence in Figure 12 with kinesin heavy chain sequences from human (Panel A), mouse (Panel B), squid (Panel C) or the portion of mouse *khcs* encoded by the anti-*khcs* GSE (Panel D).

Figure 14 shows the plating efficiency in the presence of various chemotherapeutic drugs of NIH 3T3 cells infected with the LNCX vector (indicated by crosses) or with the LNCX vector containing the anti-*khcs* GSE (indicated by dots).

Figure 15 demonstrates increased immortalization of primary mouse embryo fibroblasts by infection with the LNCX vector containing the anti-*khcs* GSE, relative to cells infected with the LNCX vector alone or uninfected (control) cells.

Figure 16 shows cDNA-PCR quantitative analysis of expression of the human *khcs* gene in various unselected and etoposide-selected human HeLa cells. Lanes a shows results for clone CS(O), lands a' for clone CX(200), lanes b for clone Σ/11(O), lanes b' for clone Σ11 (1000), lanes c for clone 6(O), lanes c' for clone 6(1000), lanes d for clone Σ20(O) and lanes d' for clone Σ20 (1000). The numbers in parentheses for each clone name indicate the concentration of etoposide (ng/ml) present in the growth media. Bands indicative of *khcs* expression are shown along with bands for β-2 macroglobulin expression as an internal control.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The invention relates to means for suppressing specific gene functions that are associated with sensitivity to chemotherapeutic drugs. The invention provides genetic suppressor elements (GSEs) that have such suppressive effect and thus confer resistance to chemotherapeutic drugs. The invention further provides methods for identifying such GSEs, as well as methods for their use.

In a first aspect, the invention provides a method for identifying GSEs that confer resistance to any chemotherapeutic drug for which resistance is possible. The GSEs identified by this method will be homologous to a gene that is associated with sensitivity to one or more chemotherapeutic drug. For purposes of the invention, the term "homologous to a gene" has two different meanings, depending on whether the GSE acts through an antisense or antigene mechanism, or rather through mechanism of interference at the protein level. In the former case, a GSE that is an antisense or antigene oligonucleotide or polynucleotide is homologous to a gene if it has a nucleotide sequence that hybridizes under physiological conditions to the gene or its mRNA transcript by Hoogsteen or Watson-Crick base-pairing. In the

latter case, a GSE that interferes with a protein molecule is homologous to the gene encoding that protein molecule if it has an amino acid sequence that is the same as that encoded by a portion of the gene encoding the protein, or that would be the same, but for conservative amino acid substitutions. In either case, as a practical matter, whether the GSE is homologous to a gene is determined by assessing whether the GSE is capable of inhibiting or reducing the function of the gene.

The method according to this aspect of the invention comprises the step of screening a total cDNA or genomic DNA random fragment expression library phenotypically to identify clones that confer resistance to a chemotherapeutic drug. Preferably, the library of random fragments of total cDNA or genomic DNA is cloned into a retroviral expression vector. In this preferred embodiment, retrovirus particles containing the library are used to infect cells and the infected cells are tested for their ability to survive in a concentration of a chemotherapeutic drug that kills uninfected cells. Preferably, the inserts in the library will range from about 100 b.p. to about 700 b.p. and more preferably, from about 200 b.p. to about 500 b.p. Most preferably, the random fragment library will be a normalized library containing roughly equal numbers of clones corresponding to each gene expressed in the cell type from which it was made, without regard for the level of expression of any gene. However, normalization of the library is unnecessary for the isolation of GSEs that are homologous to abundantly or moderately expressed genes. Once a clonal population of cells that are resistant to the chemotherapeutic drug has been isolated, the library clone encoding the GSE is rescued from the cells. At this stage, the insert of the expression library may be tested for its nucleotide sequence. Alternatively, the rescued library clone may be further tested for its ability to confer resistance to chemotherapeutic drugs in additional transfection or infection and selection assays, prior to nucleotide sequence determination. Determination of the nucleotide sequence, of course, results in the identification of the GSE. This method is further illustrated in Examples 2-5.

In a second aspect, the invention provides a method for identifying and cloning genes that are associated with sensitivity to chemotherapeutic drugs, including genes that have not been previously discovered. This is because GSEs, or portions thereof, can be used as probes to screen full length cDNA or genomic libraries to identify their gene of origin. In some cases, genes that are associated with sensitivity to chemotherapeutic drugs will turn out to be quite surprising. For example, GSEs that abrogate etoposide sensitivity are of a particularly surprising nature. The target for etoposide is topoisomerase II, a DNA unwinding enzyme. GSEs prepared from random fragments of topoisomerase II DNA do confer resistance to etoposide. Accordingly, it would be expected that most GSEs conferring etoposide resistance would be derived from DNA encoding topoisomerase II. Surprisingly, this is not the case at all. Of three etoposide resistance-conferring GSEs obtained, two were derived from previously unidentified DNA sequences. A third such GSE was derived from the kinesin heavy chain gene. Prior to this discovery, there was no suspicion that kinesin was in any way implicated in etoposide sensitivity. These results suggest that the method according to this aspect of the invention will provide much new and surprising information about the genetic basis for resistance to chemotherapeutic drugs. In addition, a kinesin-derived GSE conferring resistance to etoposide caused cellular effects suggesting that kinesin may be involved in programmed cell death. If this is indeed the case, then the method according to this aspect of the invention also provides valuable information about the genetic basis for senescence and cell death. This may have important implications for studying genes involved in development, since GSEs used to identify genes associated with chemotherapeutic drug resistance or senescence can also be expressed as transgenes in embryos to determine the role of such genes in development. The method according to this aspect of the invention and its use for studying genes identified thereby and their cellular effects are further illustrated in Examples 6-8.

In a third aspect, the invention provides a method for obtaining GSEs having optimized suppressor activity for a gene associated with sensitivity to a chemotherapeutic drug. In the method according to this aspect of the invention, an initial GSE is obtained by the method according to the first aspect of the invention. Then, the gene from which the GSE is derived is identified and cloned by the method according to the second aspect of the invention. This gene is then randomly fragmented and cloned into an expression vector, preferably a retroviral vector, to obtain a random fragment expression library derived exclusively from the gene of interest. This library is then transferred to and expressed in mammalian cells, which are selected in the presence of the appropriate chemotherapeutic drug. As a practical matter, such a library will contain a much greater variety of GSEs derived from the gene of interest than will a random fragment library prepared from total cDNA. Consequently, the likelihood of obtaining optimized GSEs, as determined by maximized chemotherapeutic drug resistance, from the single gene random fragment library approach is shown in greater detail in Example 1.

In a fourth aspect, the invention provides synthetic peptides and oligonucleotides that are capable of inhibiting the function of genes associated with sensitivity to chemotherapeutic drugs. Synthetic peptides according to the invention have amino acid sequences that correspond to amino acid sequences encoded by GSEs according to the invention. Synthetic oligonucleotides according to the invention have nucleotide sequences corresponding to the nucleotide sequences of GSEs according to the invention. Once a GSE is discovered and sequenced, and its orientation is determined, it is straightforward to prepare an oligonucleotide corresponding to the nucleotide sequence of the GSE (for antisense-oriented GSEs) or amino acid sequence encoded by the GSE (for sense-oriented GSEs). In certain embodiments, such synthetic peptides or oligonucleotides may have the complete sequence encoded by the GSE or may have only part of the sequence present in the GSE, respectively. In certain other embodiments, the peptide or

oligonucleotide may have only a portion of the GSE-encoded or GSE sequence. In such latter embodiments, undue experimentation is avoided by the observation that many independent GSE clones corresponding to a particular gene will have the same 5' or 3' terminus, but generally not both. This suggests that many GSE's have one critical endpoint, from which a simple walking experiment will determine the minimum size of peptide or oligonucleotide necessary to inhibit gene function. For peptides, functional domains as small as 6-8 amino acids have been identified for immunoglobulin binding regions. Thus, peptides or peptide mimetics having these or larger dimensions can be prepared as GSEs. For antisense oligonucleotides, inhibition of gene function can be mediated by oligonucleotides having sufficient length to hybridize to their corresponding mRNA under physiological conditions. Generally, oligonucleotides having about 12 or more bases will fit this description. Preferably, such oligonucleotides will have from about 12 to about 100 nucleotides. As used herein, the term oligonucleotide includes modified oligonucleotides having nuclease-resistant internucleotide linkages, such as phosphorothioate, methylphosphonate, phosphorodithioate, phosphoramidate, phosphotriester, sulfone, siloxane, carbonate, carboxymethylester, acetamidate, carbamate, thioether, bridged phosphoramidate, bridged methylene phosphonate and bridged phosphorothioate internucleotide linkages. The synthesis of oligonucleotides containing these modified linkages is well known in the art. (*See e.g.*, Uhlmann and Peyman, 1990, *Chem. Rev.* 90:543-584 (1990); Schneider and Banner, 1990, *Tetrahedron Lett.* 31:335). The term oligonucleotides also includes oligonucleotides having modified bases or modified ribose or deoxyribose sugars.

In a fifth aspect, the invention provides a diagnostic assay for tumor cells that are resistant to one or more chemotherapeutic drug due to absence of expression or underexpression of a particular gene. By using the methods according to the first and second aspects of the invention such a gene will be identified and cloned. To determine whether absence of expression or

underexpression of such a gene is a naturally occurring, and thus medically significant basis for chemotherapeutic drug resistance, human tumor cells can be treated with cytotoxic quantities of an appropriate chemotherapeutic drug to select for spontaneous drug resistant mutants. These mutants can then be assessed for their level of expressing of the particular gene of interest. Absence of expression or significantly reduced expression indicates a natural mechanism of chemotherapeutic drug resistance. Accordingly, such reduced or absent expression can be the basis for a diagnostic assay for tumor cell resistance to the chemotherapeutic drug or drugs of interest. A first embodiment of a diagnostic assay according to this aspect of the invention utilizes an oligonucleotide or oligonucleotides that is/are homologous to the sequence of the gene for which expression is to be measured. In this embodiment, RNA is extracted from a tumor sample, and RNA specific for the gene of interest is quantitated by standard filter hybridization procedures, an RNase protection assay, or by quantitative cDNA-PCR. (*See* Noonan *et al.*, 1990, *Proc. Natl. Acad. Sci. USA* 87: 7160-7164). In a second embodiment of a diagnostic assay according to this aspect of the invention, antibodies are raised against a synthetic peptide having an amino acid sequence that is identical to a portion of the protein that is encoded by the gene of interest. These antibodies are then used in a conventional quantitative immunoassay (*e.g.*, RIA or immunohistochemical assays) to determine the amount of the gene product of interest present in a sample of proteins extracted from the tumor cells to be tested, or on the surface or at locations within the tumor cells to be tested.

In a sixth aspect, the invention provides dominant selectable markers that are useful in gene co-transfer studies. Since GSEs according to the invention confer resistance to chemotherapeutic drugs, the presence of a vector that expresses the GSE can readily be selected by growth of a vector-transfected cell in a concentration of the appropriate cytotoxic drug that would be cytotoxic in the absence of the GSE. GSEs according to the invention are particularly well suited as dominant selectable markers because their small

size allows them to be easily incorporated along with a gene to be cotransferred even into viral vectors having limited packaging capacity.

In a seventh aspect, the invention provides *in vivo*-selectable markers that are useful both in gene therapy and in enhancing the effectiveness of chemotherapy. For gene therapy, GSEs according to the invention can be co-transferred on a vector into human CD 34^+ blood progenitor cells from a patient along with a therapeutic gene that, when expressed, will alleviate a genetic disorder. The cells can be selected *in vitro* for resistance to an appropriate chemotherapeutic drug, thereby assuring successful transfer of the GSE, and by implication, of the therapeutic gene as well. the progenitor cells containing the GSE and therapeutic gene can then be returned to the patient's circulation. Finally, the cells containing the GSE and therapeutic gene can be selected *in vivo* by administration of the appropriate chemotherapeutic drug (to which the GSE confers resistance) in a concentration that is cytotoxic to normal blood cells. In this way, those cells having the GSE and therapeutic gene will repopulate the patient's blood.

For enhancement of chemotherapy, a GSE according to the invention can be transferred alone or with another gene on an expression vector into $CD34^+$ blood progenitor cells taken from a cancer patient. *in vitro* selection of the progenitor cells harboring the GSE is then carried out, using the appropriate chemotherapeutic drug. The selected cells are then returned to the patient's circulation and allowed time to begin repopulating the blood. After an appropriate period, aggressive chemotherapy can be carried out, using much higher than ordinary concentrations of an appropriate chemotherapeutic drug (to which the GSE confers resistance), since toxic side effects to the immune system will be avoided due to GSE expression in those cells.

In either of these therapeutic contexts, it may be desirable to have the GSE expressed in the progenitor cells (and subsequently in the blood cells), only when its express is beneficial, *i.e.*, during *in vivo* selection or chemotherapy. To accomplish this, an inducible promoter can be used to

express the GSE. Then, then the appropriate inducing agent is added to the cells prior to and during *in vitro* selection and again prior to and during *in vivo* selection or chemotherapy. As long as the inducing agent is not normally present in the human body, the GSE will not be expressed by any other time.

In an eighth aspect, the invention provides a starting point for the rational design of pharmaceutical products that can counteract resistance by tumor cells to chemotherapeutic drugs. The protein sequence encoded by genes from which the GSEs were derived can be deduced from the cDNA sequence, and the function of the corresponding proteins may be determined by searching for homology with known genes or by searching for known functional motives in the protein sequence. If these assays do not indicate the protein function, it can be deduced through the phenotypic effects of the GSEs suppressing the gene. Such effects can be investigated at the cellular level, by analyzing various growth-related, morphological, biochemical or antigenic changes associated with GSE expression. The GSE effects at the organism level can also be studied by introducing the corresponding GSEs as transgenes in transgenic animals (*e.g.* mice) and analyzing developmental abnormalities associated with GSE expression. The gene function can also be studied by expressing the full-length cDNA of the corresponding gene, rather than a GSE, from a strong promoter in cells or transgenic animals, and studying the changes associated with overexpression of the gene.

Full-length or partial cDNA sequences can also be used to direct protein synthesis in a convenient prokaryotic or eukaryotic expression system, and the produced proteins can be used as immunogens to obtain polyclonal or monoclonal antibodies. These antibodies can be used to investigate the protein localization and as specific inhibitors of the protein function, as well as for diagnostic purposes. In particular, antibodies raised against a synthetic peptide encoded by part of the complement of the sequence of the GSE anti-*khcs*, or the corresponding region of the human KHCS protein should be particularly useful, as should antibodies raised against an amino acid sequence encoded by part of the VPA or VP9-11 GSEs (see Figures 9-11).

Understanding the biochemical function of a gene involved in drug sensitivity is likely to suggest pharmaceutical means to stimulate or mimic the function of such a gene and thus augment the cytotoxic response to anticancer drugs. For example, if the gene encodes an enzyme producing a certain compound, such a compound can be synthesized chemically and administered in combination with cytotoxic drugs. If a pharmaceutical approach is not apparent from the protein function, one may be able to upmodulate gene expression at the level of transcription. This can be done by cloning the promoter region of the corresponding gene and analyzing the promoter sequence for the presence of *cis* elements known to provide the response to specific biological stimulators.

The most straightforward way to increase the expression of a drug sensitivity gene, identified through the GSE approach, would be to insert a full-length cDNA for such a gene into a retroviral vector. Such a vector, in the form of a recombinant retrovirus, will be delivered to tumor cells *in vivo*, and, upon integration, would sensitize such cells to the effects of the corresponding chemotherapeutic drug. A similar strategy for selective delivery of a drug-sensitivity gene into rat brain tumors, followed by curative treatment with the appropriate drug, was reported by Culver *et al.* (1992, *Science* 256: 1550-1552). The selective delivery to tumor cells can be accomplished on the basis of the selectivity of retrovirus-mediated transduction for dividing cells. Alternatively, the selectivity can be achieved by driving the expression of the drug sensitivity gene from a tissue-or tumor-specific promoter, such as, for example, the promoter of the carcinoembryonic antigen gene.

The protein structure decided from the cDNA sequence can also be used for computer-assisted drug design, to develop new drugs that affect this protein in the same manner as the known anticancer drugs. The purified protein, produced in a convenient expression system, can also be used as the critical component of *in vitro* biochemical screen systems for new compounds with anticancer activity. Accordingly, mammalian cells that express chemotherapeutic drug resistance-conferring GSEs according to the invention

are useful for screening compounds for the ability to overcome drug resistance.

The following examples are intended to further illustrate certain preferred embodiments of the invention and are not limiting in nature.

5

Example 1

Development of GSEs For Human Topoisomerase II

Topoisomerase II is a DNA unwinding enzyme that serves as a target for many anti-cancer drugs, including etoposide, doxorubicin and amsacrine. The enzyme normally acts by double-strand DNA cleavage, followed by strand passage and relegation of the breaks. Anti-cancer drugs cause trapping of the enzyme in complexes having double-strand breaks held together by the enzyme, thereby leading to lethal damage in replicating cells. Some cell lines that are resistant to anti-cancer drugs that interact with topoisomerase II have decreased expression of this enzyme.

Random fragment selection of GSEs requires transfer of the expression library into a very large number of recipient cells. Therefore, to prepare a random fragment library containing GSEs to topoisomerase II, the efficient retroviral vector system was chosen. Overlapping cDNA clones spanning the entire coding sequence for topoisomerase II were mixed and randomly fragmented to 250-350 bp fragments by DNase I in the presence of Mn^{++} ions and fragment termini were filled in with T4 DNA polymerase and Klenow fragment of DNA polymerase I. After ligation with a synthetic adaptor providing translation initiation and termination codons, the fragment mixture was amplified by PCR, using adaptor-derived primers. The amplified mixture was cloned with the LNCX retroviral vector which contains a *neo* gene. (*see* Miller and Rosman, 1989, *Biotechniques* 7: 980-986).

A random fragment library containing 20,000 independent clones was obtained, and was used to transfect amphotropic and ecotropic virus-packaging cell lines derived from NIH 3T3 cells, to effect ping-pong replication-mediated amplification of the virus (*see, e.g.*, Bodine *et al.*, 1990, *Proc. Natl. Acad. Sci.*

USA 87: 3738-3742). This resulted in a random fragment expression library (RFEL), a set of recombinant retroviruses containing a representative mixture of inserts derived from topoisomerase II gene sequences.

The uniformity of sequence representation in RFEL was monitored as follows. NIH 3T3 cells were infected with virus-containing supernatant, followed 24 hours later by PCR amplification of integrated proviral insert sequences in the presence of [^{32}P] alpha-dNTP. An aliquot of the PCR-amplified mixture was subjected to gel electrophoresis to establish the absence of predominant bands. Another aliquot was used as a probe for a Southern blot of topoisomerase II cDNA digested with several frequently cutting restriction enzymes. A representative sequence mixture was obtained, as evidenced by the absence of a predominant band in the first test, and uniform hybridization to all fragments in the second test.

RFEL was then used to infect HeLa cells, and the infectants were selected with G418. Colonies of G418-resistant cells, having about 50-70 cells each, were then exposed to etoposide at a concentration of 200 μg/ml. Approximately 50 of 10,000 G418-resistant colonies were etoposide resistant, compared to a frequency of $< 10^{-4}$ when insertless retroviruses were used as a control. Cell lines were isolated from etoposide-resistant colonies. Amphotropic and ecotropic packaging cell lines producing RFEL were also selected for etoposide resistance. Virus from etoposide resistant packaging cell lines was used to infect HeLa cells, which were then selected from G418. G418-resistant infectants were challenged with three topoisomerase II-interactive anticancer drugs: etoposide, teniposide and amsacrine. A high proportion of infected cells were resistant to all three drugs, thus demonstrating that etoposide selection of mouse packaging cell lines has led to the generation of GSEs active in both human and mouse cells. These infectants were also used to establish cells lines. RFEL-derived inserts were recovered from etoposide resistant cell lines by PCR and recloned into LNCX vector. The newly-derived clones were then individually tested for the ability

to confer resistance to etoposide upon transfection into HeLa cells, to confirm the GSE activity of the corresponding inserts.

Sequence analysis of 26 different isolated clones revealed that 16 of them were inserted in antisense and 10 in sense orientation. Of the 12 GSEs confirmed, 7 were sense and 5 antisense, as shown in Table I. The sequences of the confirmed GSEs are shown in Figure 1. The sense-oriented inserts of the confirmed GSEs encode 37-99 amino acid long topo II-derived peptides, initiating either from the ATG codon provided by the adaptor, or from an internal ATG codon with the open reading frame of Topoisomerase II, located close to the 5' end of the insert in an appropriate context for translation initiation. Four of the confirmed antisense GSEs come from the 3' third of the cDNA and one from the 5' end of cDNA, including the translation start site. Of the sense-oriented GSEs, five were derived from the central portion of the protein that includes the active site tyrosine-804 that covalently binds to DNA and the "leucine zipper" region involved in dimerization of Topoisomerase II. One GSE peptide is derived from the region near the N-terminus and another from the region near the C-terminus of the protein; no known functional sites are associated with either segment.

These results demonstrate the GSEs that act according to multiple mechanisms to confer etoposide resistance can be prepared from a random fragment library of DNA encoding topoisomerase II. In addition, these results show that GSEs produced from one mammalian species can be active in another mammalian species.

Table I
Confirmed Topoisomerase II-Derived GSE

Clones	Orientation (Sense/Antisense)	Position in cDNA[2]	Position of peptide[b]
2V	Antisense	-18 - 145	
Σ11	Sense	393 - 605	134 - 201
6	Sense	2352 - 2532	808 - 844
5	Sense	2511 - 2734	846 - 911
Σ28	Sense	2603 - 2931	879 - 977
Σ2	Antisense	3150 - 3343	
Σ20	Antisense	3486 - 3692	
39	Antisense	3935 - 4127	
12S	Sense	4102 - 4343	1368 - 1447
ΣVPs2	Sense	2494 - 2834	846 - 944
Σ8	Antisense	4123 - 4342	
ΣVM	Sense	2501 - 2670	846 - 890

[a] Position in the cDNA sequence of topoisomerase II; residues numbered as in Tsai-Pflugfelder *et al.*, 1988, *Proc. Natl. Acad. Sci. USA* 85: 7177-7181.

[b] Position of the peptide encoded by sense-oriented GSEs in the amino acid sequence of topoisomerase II; translation assumed to initiate from the first ATG codon in the correct open reading frame.

Example 2

Generation Of A Normalized Random Fragment cDNA Library In A Retroviral Vector

As shown in Figure 2 a normalized cDNA population was prepared using a modification of the protocol of Patanjali *et al.* (1991, *Proc. Natl. Acad. Sci. USA* 88: 1943-1947). Poly(A)$^+$ RNA was extracted from NIH 3T3 cells. To obtain mRNAs for different genes expressed at various stages of the cell growth, one half of the RNA was isolated from a rapidly growing culture and the other half from quiescent cells that had reached complete monolayer confluency. To avoid overrepresentation of the 5'-end sequences in a

randomly primed cDNA population, RNA was fragmented by boiling to an average size range 600-1,000 nucleotides. These RNA fragments were then used for preparing randomly primed double-stranded cDNA. This randomly primed cDNA was then ligated to a synthetic adaptor providing ATG codons in all three possible reading frames and in a proper context for translation initiation. The structure of the adaptor (see Figure 3) determined its ligation to the blunt-ended fragments of the cDNA in such a way that each fragment started from initiation codons independently from its orientation. The adaptor was not supplied with termination codons in the opposite strand since the cloning vector pLNCX, contained such codons immediately downstream of the cloning site. This vector has been described by Miller and Rosman (1989, *Biotechniques* 7: 980-986). The litigated mixture was amplified by PCR, using the "sense" strand of the adaptor as a PCR primer, in contrast to the method of Patanjali et al., which utilized cloning the initial cDNA preparation into a phage vector and then using vector-derived sequences as PCT primers to amplify the cDNA population. The PCRs were carried out in 12 separate reactions that were subsequently combined, to minimize random over- or under-amplification of specific sequences and to increase the yield of the product. The PCR-amplified mixture was size-fractionated by gel electrophoresis, and 200-500 bp fragments were selected for subsequent manipulations in contrast to Patanjali's fragment size range of from 400 to 1,600 bp.

For normalization, the cDNA preparation was denatured and reannealed, using different time points, as described by Patanjali *et al.*, *supra*, and shown in Figure 2, for reannealing. The single-stranded and double-stranded DNAs from each reannealed mixture were separated by hydroxyapatite chromatography. The single-stranded DNA fractions from each time point of reannealing were PCT-amplified using the adaptor-derived primer and analyzed by Souther hybridization for the relative abundance of different mRNA sequences. The fraction that contained similar proportions of tubulin, *c-myc* and *c-fos* cDNA sequences (see Figure 2), corresponding to

medium- and low-expressed genes, respectively, was used for the library preparation.

The normalized cDNA preparation was cloned into the *Cla*I site of the MoMLV-based retroviral vector pLNCX, which carries the *neo* (G418 resistance) gene, transcribed from the promoter contained in the retroviral long terminal repeat (LTR), and which expresses the inserted sequence from a strong promoter of the cytomegalovirus (CMV) (see Figure 3). The ligation mixture, divided into five portions, was used for five subsequent large-scale transformations of *E. coli*. The transformed bacteria was plated on the total of 500 agar plates (150 mm in diameter) and the plasmid population (18 mg total) was isolated from the colonies washed off the agar. A total of approximately 5 x 10^7 clones were obtained, more than 60% of which carried the inserts of normalized cDNA, as estimated by PCR amplification of inserts from 50 randomly picket colonies. These results demonstrate the feasibility of generating a normalized cDNA library of as many as 3 x 10^7 recombinant clones in a retroviral plasmid expression vector.

Example 3

Transduction Of A Retroviral Random Fragment Library Into Virus-Packaging Cell Lines and NIH 3T3 Cells

The plasmid library prepared according to Example 2 was converted into a mixture of retroviral particles by transfection into virus-packaging cells (derivatives of NIH 3T3) that express retroviral virion proteins. Examples of such cell lines have been described by Markowitz *et al.* (1988, *Virology* 167: 400-406). Ecotropic and amphotropic virus-packaging cell lines, GP + E86 and GP + envAm12, respectively, were mixed at 1:1 ratio, and 10^7 cells of this mixture were transfected with the plasmid library under standard calcium phosphate coprecipitation conditions. This transfection resulted in the packaging and secretion of ecotropic and amphotropic virus particles, which rapidly spread through the packaging cell population, since ecotropic viruses

are capable of infecting amphotropic packaging cells and vice versa. The yield of the virus, as measured by the number of G418-resistant colonies obtained after the infection of NIH 3T3 cells, reached 10^5 infectious units per 1 ml of media during the stage of transient transfection (1-3 days), then decreased (4-8 days) and then rapidly increased due to the expression of proviral genomes that became stably integrated in most of the packaging cells. The yield of the virus 9-12 days after transfection reached $> 10^6$ per 1 ml of media supernatant. At this stage, the library showed fairly even representation of different fragments, but at later stages individual virus-producing clones began to predominate in the population, leading to uneven representation of cDNA-derived inserts. The uniformity of sequence representation in the retroviral population was monitored by rapid extraction of DNA from cells infected with the virus-containing supernatant, followed by PCR amplification of inserts. The inserts were analyzed first by the production of a continuous smear in ethidium-bromide stained agarose gel and then by Southern hybridization with different probes, including topoisomerase II, *c-myc* and tubulin. As long as each gene was represented by a smear of multiple fragments, the representativity of the library was considered to be satisfactory.

In other experiments, for transducing the random-fragmented normalized cDNA library into NIH 3T3 cells, without loss of representativity, NIH 3T3 cells were infected either with a virus produced at the transient stage of transfection (days 1-3), or with the high-titer virus collected 10-12 days after transfection. In the latter case, 100 ml of viral suspension contained more than 10^8 infectious units. In the case of the "transient"virus, NIH 3T3 cells were infected with at least 10^7 recombinant retroviruses by using 500 ml of media from virus-producing cells (five rounds of infection, 100 ml of media in each). These results demonstrate the feasibility of converting a large and complex random fragment library into retroviral form and delivering it to a non-packaging cell line without loss of complexity.

Example 4

Isolation of GSEs Conferring Resistance To The Chemotherapeutic Drug Etoposide

The overall scheme for the selection of GSEs conferring etoposide resistance is illustrated in Figure 4. This selection was carried out directly on virus-producing packaging cells, in the expectation that cells whose resistant phenotype is caused by the GSE expression will produce virus particles carrying such a GSE. The mixture of amphotropic and ecotropic packaging cells was transfected with the cDNA library in the LNCX vector, prepared according to Example 2 and the virus was allowed to spread through the population for 9 days. Analysis of a small part of the population for G418 resistance showed that practically 100% of the cells carried the *neo*-containing provirus. The cells were then exposed to 350 ng/ml etoposide for 15 days and then allowed to grow without drug for two more weeks. No difference was observed between the numbers of colonies obtained in the experiment and in the control (uninfected cells or cells infected with the insert-free LNCX virus) after etoposide selection. The virus present in the media supernatant of the surviving cells was then used to infect NIH 3T3 cells followed by etoposide selection using essentially the same protocol. NIH 3T3 cells infected with the library-derived virus produced by packaging cells that were selected with etoposide showed a major increase in the number of etoposide-resistant cells relative to the control cells infected with the insert-free LNCX virus, indicating the presence of biologically active GSEs in the preselected virus population (see Figure 5A).

The proviral inserts contained in the etoposide-selected NIH 3T3 cells were analyzed by PCR. This analysis (see Figure 5B) showed an enrichment for specific fragments, relative to the unselected population of the infected cells. Individual PCR-amplified fragments were recloned into the LNCX vector in the same position and orientation as in the original plasmid, as illustrated in Figure 6. A total of 42 proviral inserts, enriched after etoposide selection, were thus recloned, and tested either in batches or individually for

the ability to confer increased etoposide resistance after retroviral transduction into NIH 3T3 cells. Three non-identical clones were found to induce etoposide resistance, indicating that they contained biologically active GSEs. Etoposide resistance induced by these clones is illustrated in Figures 7 and 8. These GSEs were named anti-*khcs*, VPA and VP9-11.

The ability of one of these GSEs (anti-*khcs*) to induce etoposide resistance was further documented by using the isopropyl β-D-thiogalactopyranoside (IPTG)-inducible promoter/activator system, as described by Baim *et al.* (1991, *Proc. Natl. Acad. Sci. USA* 88: 5072-5076). The components of this system include an enhancer-dependent promoter, combined in cis with multiple repeats of the bacterial *lac* operator, and a gene expressing LAP265, an artificial regulatory protein derived from the *lac* repressor and a mammalian transcriptional activator. The anti-*khcs* GSE was cloned into the plasmic pX6.CLN, which contains the inducible promotor used by Baim *et al.*, *supra*, (a gift of Dr. T. Shenk) which expresses the inserts from an enhancerless SV40 early gene promoter supplemented with 21 repeats of the *lac* operator sequence. The resulting plasmid, which contains no selectable markers, was co-transfected into NIH 3T3 cells together with the LNCX plasmid carrying the *neo* gene. The mass population of G418-selected transfectants, along with control cells transfected with the insert-free vector, was exposed to increasing concentrations of etoposide, in the presence or in the absence of 5 mM IPTG. Even though the co-transfection protocol usually leads to the integration of the GSE in only a fraction of the G418-resistant cells, transfection with anti-*khcs* resulted in a clearly increased etoposide resistance, which was dependent on IPTG (see Figure 8).

Example 5

Sequence Analysis of GSEs Conferring Resistance To The Chemotherapeutic Drug Etoposide

The GSEs anti-*khcs*, VPA, and VP9-11, cloned as described in Example 4, were sequenced by the standard dideoxy sequencing procedure, and the

deduced sequences are shown in Figure 9-11. The nucleotide sequences of the "sense" and "antisense" strands, as well as amino acid sequences of the peptides encoded by these strands, were analyzed for homology to the nucleic acid and protein sequences present in the National Center for Biotechnology Information data base, using the BLAST network program for homology search. No significant homology with any sequence was detected for GSEs VPA and VP9-11. In contrast, the sequence corresponding to the "antisense" strand of the anti-*khcs* GSE, showed strong homology with several genes encoding the heavy chain of kinesins, a family of microtubule motor proteins involved in intracellular movement of organelles or macromolecules along the microtubules of eukaryotic cells. This protein family has been reviewed by Endow, 1991, *Trends Biochem. Sci.* 16: 221-225). The highest homology was found with the human kinesin heavy chain (KHC) gene, as described by Navone *et al.*, 1992, *J. Cell Biol.* 117: 1263-1275). Anti-*khcs* therefore encodes antisense RNA for a mouse *khc* gene, which we term *khcs* for *khc* associated with *s*ensitivity (to drugs) or *s*enescence. We refer to the kinesin molecule, formed by the associate of the Khcs protein with kinesin light chains, as kinesin-S, to distinguish it from the other kinesins present in mammalian cells. These results demonstrate that chemotherapeutic drug selection for GSEs can lead to the discovery of novel genetic elements, and can also reveal roles of genes in drug sensitivity that had never before been suspected.

Example 6

Cloning And Analysis Of The Gene From Which Anti-*khcs* GSE Gene Was Derived

The anti-*khcs* GSE isolated in Example 4 was used as a probe to screen 400,000 clones from each of two cDNA libraries in the lambda gt10 vector. These libraries were prepared by conventional procedures from the RNA of mouse BALB/c 3T3 cells, either unsynchronized or at Go -> G1 transition, as described by Lau and Nathans (1985, *EMBO J.* 4: 3145-3151 and 1987, *Proc. Natl. Acad. Sci. USA* 84: 1182-1186, a gift of Dr. Lau). Screening of the first

library yielded no hybridizing clones, but two different clones from the second library were found to contain anti-*khcs* sequences. These clones were purified and sequenced. Sequence analysis showed that we have isolated the bulk of the mouse *khcs* cDNA, corresponding to 796 codons (the full-length human KHC cDNA encodes 963 amino acids). This sequence is shown in Figure 12. The missing 5' and 3' terminal sequences are currently being isolated using the "anchored PCR" technique, as described by Ohara *et al.* (1989, *Proc. Natl. Acad. Sci. USA* 86: 5763-5677).

The dot-matrix alignment of the sequenced portion of the *khcs* protein with previously cloned KHC proteins from the human (*see* Navone *et al.*, 1992, *J. Cell. Biol.* 117:1263-1275), mouse (*see* Kato, 1991, *J. Neurosci.* 2: 704-711) and squid (*see* Kosik *et al.*, 1990, *J. Biol. Chem.* 265: 3278-3283) is shown in Figure 13. The portion corresponding to the anti-*khcs* GSE, is shown in Figure 9. The *khcs* gene is most highly homologous to the human gene (97% amino acid identity), suggesting that the human KHC (KHCS) gene is functionally equivalent to the mouse *khcs*. The alignment also shows that the anti-*khcs* GSE corresponds to the region which is the most highly diverged between different kinesins. This result suggests that the anti-*khcs* GSE is an antisense-oriented GSE fragment with an inhibitory effect specific to kinesin-S and not to the other mouse kinesins. This suggests the possibility that suppression of the other members of the kinesin family would have a detrimental effect on cell growth, thus resulting in selective isolation of the most specific sequence within the *khcs* gene.

Example 7

Assessment Of Drug Cross-Resistance Conferred By Resistance To Etoposide

To determine the spectrum of drugs to which the anti-*khcs* GSE would confer resistance, we have developed a stable population of ecotropic packaging cells producing the LNCX virus with the anti-*khcs* insert. This virus was used to infect NIH 3T3 cells. Two days after infection, the cells were

analyzed for resistance to several different drugs, relative to control cells infected with the LNCX vector virus, using the standard plating efficiency assay. Figure 14 shows one out of three sets of experiments carried out with different drugs by this assay. Cells infected with the anti-*khcs* virus showed a clear increase in their resistance to etoposide and Adriamycin relative to control NIH 3T3 cells infected with the control LNCX virus. No changes in resistance were observed with colchicine, cisplatin, camptothecin, or actinomycin D. These latter results remain preliminary, however, because this assay, analyzing total unselected virus-infected populations is relatively insensitive, compared with analysis of highly expressing individually-selected clones. Overall, these results demonstrate that selection of GSEs that confer resistance to one chemotherapeutic drug can result in obtaining GSEs that confer resistance to additional chemotherapeutic drugs.

Example 8

Assessment of Cellular Effects Of GSEs That Confer Resistance To Etoposide

The virus carrying the anti-*khcs* GSE was tested for the ability to increase the life span of primary mouse embryo fibroblasts (MEF). MEF were prepared form 10 day old mouse embryos by a standard trypsinization procedure and senescent cells were frozen at different passages prior to crisis. Senescent MEF, two weeks before crisis, were infected with recombinant retroviruses carrying LNCX vector either without an insert or with anti-*khcs*. Figure 15 shows MEF cell colonies two weeks after crisis. Relative to uninfected MEF cells, or cells infected with a control LNCX virus, cells infected with the anti-*khcs* showed a great increase in the proportion of cells surviving the crisis. Post-crisis cells infected with the anti-*khcs* virus showed no microscopically visible features of neoplastic transformation. These results indicate that anti-*khcs* promotes the immortalization of normal senescent fibroblasts. These results suggest that the normal function of kinesin-S may be associated with the induction of programmed cell death occurring after exposure to certain cytotoxic drugs or in the course of cellular senescence.

These results also indicate that isolation of GSEs that confer resistance to chemotherapeutic drugs can provide insight into the cellular genes and processes involved in cell growth regulation.

Example 9

Assessment Of The Role Of Decreased *khcs* Gene Expression In Naturally Occurring Mechanisms Of Drug Resistance

To test whether decreased *khcs* gene expression is associated with any naturally occurring mechanisms of drug resistance, an assay was developed for measuring *khcs* mRNA levels by cDNA-PCR. This assay is a modification of the quantitative assay described by Noonan *et al.*(1990, *Proc. Natl. Acad. Sci. USA* 87: 7160-7164) for determining *mdr-1* gene expression. The oligonucleotide primers had the sequences AGTGGCTGGAAAACGAGCTA [SEQ. ID. NO. 19] and CTTGATCCCTTCTGGTTGAT [SEQ. ID. NO. 20]. These primers were used to amplify a 327 bp segment of mouse *khcs* cDNA, corresponding to the anti-*khcs* GSE. These primers efficiently amplified the mouse cDNA template but not the genomic DNA, indicating that they spanned at least one intron in the genomic DNA. Using these primers, we determined that *khcs* mRNA is expressed at a higher level in the mouse muscle tissue than in the kidney, liver or spleen.

In another experiment a pair of primers amplifying a homologous segment of the human KHCS cDNA was selected, based on the reported human KHC sequence published by Navone *et al.* (1992, *J. Cell. Biol.* 117: 1263-1275). The sequences of these primers are AGTGGCTTGAAAATGAGCTC [SEQ. ID. NO. 21] and CTTGATCCCTTCTGGTAGATG [SEQ. ID. NO. 22], and they amplify a 327 bp cDNA fragment. These primers were used to test for changes in the KHCS gene expression in several independently isolated populations of human HeLa cells, each selected for spontaneously acquired etoposide resistance, β_2-microglobulin cDNA sequences were amplified as an internal control. Figure 16 shows the results of the cDNA-PCR assay on the following populations:

CX(0), HeLa population infected with the LNCX vector virus and selected with G418; CX (200), the same cells selected for resistance to 200 ng/ml etoposide; Σ11(O), 6(O) and Σ21(O), populations obtained after infection of HeLa cells with recombinant retroviruses carrying different GSEs derived from topoisomerase α cDNA, as described in Example 1, and selected with G418:Σ11 (1000), 6(1000) and Σ21(1000), the same populations selected for resistance to 1 μg/ml etoposide. As shown in Figure 16, the yield of the PCR product specific for the *khcs* gene was significantly lower in each of the etoposide-selected populations than in the control cells. This result indicates that a decrease in the *khcs* gene expression is a common natural mechanism for drug resistance.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Board of Trustees of the University of Illinois
(B) STREET: 352 Henry Administration Building, 506 South Wright Street
(C) CITY: Urbana
(D) STATE: Illinois
(E) COUNTRY: USA
(F) POSTAL CODE (ZIP): 61801
(G) TELEPHONE:
(H) TELEFAX:

(ii) TITLE OF INVENTION: Genes And Genetic Elements Associated With Sensitivity To Chemotherapeutic Drugs

(iii) NUMBER OF SEQUENCES: 22

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(v) CURRENT APPLICATION DATA:
APPLICATION NUMBER: PCT/US94/

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 164 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GTGTCTGGGC GGAGCAAAAT ATGTTCCAAT TGTGTTTTCT TTTGATAGAT TCTTTCAACA 60

GACAGTCTTT TCTTAGCATC TTCATTTTTC TTTATTTTGT TGACTTGCAT ATTTTCATTT 120

ACAGGCTGCA ATGGTGACAC TTCCATGGTG ACGGTCGTGA AGGG 164

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 213 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TGAAAAGATG TATGTCCCAG CTCTCATATT TGGACAGCTC CTAACTTCTA GTAACTATGA 60

TGATGATGAA AAGAAAGTGA CAGGTGGTCG AAATGGCTAT GGAGCCAAAT TGTGTAACAT 120

ATTCAGTACC AAATTTACTG TGGAAACAGC CAGTAGAGAA TACAAGAAAA TGTTCAAACA 180

GACATGGATG GATAATATGG GAAGAGCTGG TGA 213

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 181 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GCCCATTGGT CAGTTTGGTA CCAGGCTACA TGGTGGCAAG GATTCTGCTA GTCCACGATA 60

CATCTTTACA ATGCTCAGCT CTTTGGCTCG ATTGTTATTT CCACCAAAAC ATGATCACAC 120

GTTGAAGTTT TTATATGATG ACAACCAGCG TGTTGAGCCT GAATGGTACA TTCCTATTAT 180

T 181

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 224 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TGAATGGTAC ATTCCTATTA TTCCCATGGT GCTGATAAAT GGTGCTGAAG GAATCGGTAC 60

TGGGTGGTCC TGCAAAATCC CCAACTTTGA TGTGCGTGAA ATTGTAAATA ACATCAGGCG 120

TTTGATGGAT GGAGAAGAAC CTTTGCCAAT GCTTCCAAGT TACAAGAACT TCAAGGGTAC 180

TATTGAAGAA CTGGCTCCAA ATCAATATGT GATTAGTGGT GAAG 224

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 329 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TGCGTGAAAT TGTAAATAAC ATCAGGCGTT TGATGGATGG AGAAGAACCT TTGCCAATGC 60

TTCCAAGTTA CAAGAACTTC AAGGGTACTA TTGAAGAACT GGCTCCAAAT CAATATGTGA 120

TTAGTGGTGA AGTAGCTATT CTTAATTCTA CAACCATTGA AATCTCAGAG CTTCCCGTCA 180

GAACATGGAC CCAGACATAC AAAGAACAAG TTCTAGAACC CATGTTGAAT GGCACCGAGA 240

AGACACCTCC TCTCATAACA GACTATAGGG AATACCATAC AGATACCACT GTGAAATTTG 300

TTGTGAAGAT GACTGAAGAA AAACTGGCA 329

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 194 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CACTCTTTTC AGTTTCCTTT TCGTTGTCAC TCTCTTCATT TTCTTCTTCA TCTGGAACCT 60

TTTGCTGGGC TTCTTTCCAG GCCTTCACAG GATCCGAATC ATATCCCCTC TGAATCAGAA 120

CTTTAATTAA TTCTTTCTTA GGCTTATTTT CAATGATTAT TTTGCCATCT ATTTTCTCAT 180

AGATAAAGCG AGCC 194

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 206 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TCTGCCTCTG CTTTCATTTC TATGGTTATT CGTGGAATGA CTCTTTGACC ACGCGGAGAA 60

GGCAAAACTT CAGCCATTTG TGTTTTTTTC CCCTTGGCCT TCCCCCCTTT CCCAGGAAGT 120

CCGACTTGTT CATCTTGTTT TTCCTTGGCT TCAACAGCCT CCAATTCTTC AATAAATGTA 180

GCCAAGTCTT CTTTCCACAA ATCTGA 206

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 194 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GACACGACAC TTTTCTGTGG TTTCAGTTCT TTGTTACTAA GTTTTGGGGA AGTTTTGGTC 60

TTAGGTGGAC TAGCATCTGA TGGGACAAAA TCTTCATCAT CAGTTTTTTC ATCAAAATCT 120

GAGAAATCTT CATCTGAATC CAAATCCATT GTGAATTTTG TTTTTGTTGC TGCTCTCGGT 180

GGCTCTGTTT CTCG 194

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 242 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CTGAAACCAC AGAAAAGTGT CGTGTCAGAC CTTGAAGCTG ATGATGTTAA GGGCAGTGTA 60

CCACTGTCTT CAAGCCCTCC TGCTACACAT TTCCCAGATG AAACTGAAAT TACAAACCCA 120

GTTCCTAAAA AGAATGTGAC AGTGAAGAAG ACAGCAGCAA AAAGTCAGTC TTCCACCTCC 180

ACTACCGGTG CCAAAAAAAG GGCTGCCCCA AAAGGAACTA AAAGGGATCC AGCTTTGAAT 240

TC 242

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 341 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

AACCAGCGTG TTGAGCCTGA ATGGTACATT CCTATTATTC CCATGGTGCT GATAAATGGT 60

GCTGAAGGAA TCGGTACTGG GTGGTCCTGC AAAATCCCCA ACTTTGATGT GCGTGAAATT 120

GTAAATAACA TCAGGCGTTT GATGGATGGA GAAGAACCTT TGCCAATGCT TCCAAGTTAC 180

AAGAACTTCA AGGGTACTAT TGAAGAACTG GCTCCAAATC AATATGTGAT TAGTGGTGAA 240

GTAGCTATTC TTAATTCTAC AACCATTGAA ATCTCAGAGC TTCCCGTCAG AACATGGACC 300

CAGACATACA AAGAACAAGT TCTAGAACCC ATGTTGAATG G 341

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 220 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

AATTCAAAGC TGGATCCCTT TTAGTTCCTT TTGGGGCAGC CCTTTTTTTG GCACCGGTAG 60

TGGAGGTGGA AGACTGACTT TTTGCTGCTG TCTTCTTCAC TGTCACATTC TTTTTAGGAA 120

CTGGGTTTGT AATTTCAGTT TCATCTGGGA AATGTGTAGC AGGAGGGCTT GAAGACAGTG 180

GTACACTGCC CTTAACATCA TCAGCTTCAA GGTCTGACAC 220

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 170 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GTGTTGAGCC TGAATGGTAC ATTCCTATTA TTCCCATGGT GCTGATAAAT GGTGCTGAAG 60

GAATCGGTAC TGGGTGGTCC TGCAAAATCC CCAACTTTGA TGTGCGTGAA ATTGTAAATA 120

ACATCAGGCG TTTGATGGAT GGAGAAGAAC CTTTGCCAAT GCTTCCAAGT 170

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AATCATCGAT GGATGGATGG 20

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CCATCCATCCATCGATGATTAAA 23

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 327 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CTTGATCCCT TCTGGTTGAT GCCAGAAGCT CTTCCTGATC CAGCATTTGT ATCTTCAATT 60

TCTCTACCAA TTGGCTTTGT TGGTTAATCT CTTCATCCTT GTCATCAAGT TGTTTATACA 120

ATTTAGCAAG TTCTTCTTCA CACTTTCTTC TTTCAGCATC GGTAAAACTA CCAGCCATTC 180

CGACTGCAGC AGCTGGTTTA TCACTGGTAA TAGCAATATC TTTATCCGCT GTGAAGGCTT 240

CCAAATTAGC TTTCTCTTTG TCAAACTGCT CATCAATAGG CACTGTCTCC CCGTTACGCC 300

AACGGTTTAG CTCGTTTTCC AGCCACT 327

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 250 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CCGACCGGGA GCGGGAGAAG GAGCGGGAGC GGGAGCAGCG GGAGAAGGAG CGGGAGAAGG 60

AGCTGGAGCG CGACGGGAGA AGGAACGGGA GCGCGAGCTG GAGCGGCAGC GGGAGCAGCG 120

GGCGAGGGAG AAGGAGCTGC TGGCTGCCAA GGCCTTAGAG CCCACCACCT TCCTGCCTGT 180

GGCCGAGCTG CACGGACTCC GAGGTCACAC CACGGAGGAG CGGCCCAAGC CCTCGGAGCA 240

GCTGACCCCA 250

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 208 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CTCAGAGGTG ATCCTCTCGG AGTCGAGCTC AGGAGAAGGA GTCCCCTTCT TTGAGACTTG 60

GATGCAGACC TGCATGTCCG AGGAGGGCAA GATTTTGAAC CCTGACCATC CCTGCTTCCG 120

CCCTGACTCC ACCGAAGTCG AGTCCTTGGT GGCCCTGCTC AACAACTCTT CAGAGATGAA 180

GCTAGTACAG ATGAAGTAGC ACGAGGCC 208

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2389 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CGACAAACAT CATCTGGGAA GACCCACACG ATGGAGGGTA AACTTCATGA TCCAGAAGGC 60

ATGGGAATTA TTCCAAGAAT AGTGCAAGAT ATTTTTAATT ATATTTACTC CATGGATGAA 120

AATTTGGAAT TTCATATTAA GGTTTCATAT TTTGAAATAT ATTTGGATAA GATAAGGGAC 180

TTGTTAGATG TTTCAAAGAC TAACCTTTCA GTCCATGAAG ACAAAAACCG TGTTCCCTAT 240

GTAAAGGGGT GCACAGAACG TTTCGTGTGT AGTCCAGATG AAGTCATGGA TACCATAGAT 300

GAAGGGAAAT CCAACAGAGA TGTCGCAGTT ACAAATATGA ATGAACATAG CTCTAGGAGC 360

CACAGCATAT TTCTTATTAA TGTAAAACAA GAGAATACAC AAACGGAACA GAAACTCAGT 420

GGAAAGCTTT ATCTGGTTGA TTTAGCTGGC AGTGAGAAGG TTAGTAAGAC TGGGGCTGAA 480

GGTGCTGTGC TGGATGAAGC TAAGAACATC AAGAAGTCAC TTTCTGCACT TGGAAATGTC 540

ATTTCTGCTT TGGCAGAGGG CAGTACCTAT GTTCCTTATC GAGATAGTAA AATGACCAGA 600

ATTCTTCAAG ATTCATTAGG TGGCAACTGT AGGACCACTA TTGTCATATG CTGCTCTCCA 660

TCATCATACA ATGAGTCTGA GACAAAGTCA ACACTCCTCT TTGGTCAAAG GGCCAAAACA 720

ATTAAGAACA CAGTCTGTGT CAATGTAGAG TTAACTGCAG AGCAGTGGAA AAAGAAGTAT 780

GAAAAAGAAA AGGAAAAAAA TAAGACTCTA CGGAACACTA TTCAGTGGCT GGAAAACGAG 840

CTAAACCGTT GGCGTAACGG GGAGACAGTG CCTATTGATG AGCAGTTTGA CAAAGAGAAA 900

GCTAATTTGG AAGCCTTCAC AGCGGATAAA GATACTGCTA TTACCAGTGA TAAACCAGCT 960

GCTGCAGTCG GAATGGCTGG TAGTTTTACC GATGCTGAAA GAAGAAAGTG TGAAGAAGAA 1020

CTTGCTAAAT TGTATAAACA GCTTGATGAC AAGGATGAAG AGATTAACCA ACAAAGCCAA 1080

TTGGTAGAGA AATTGAAGAC ACAAATGCTG GATCAGGAAG AGCTTCTGGC ATCAACCAGA 1140

AGGGATCAAG ATAATATGCA AGCTGAACTG AATCGCCTCC AAGCAGAAAA TGATGCTTCT 1200

AAAGAAGAAG TCAAAGAAGT TTTACAGGCC TTAGAGGAAC TGGCTGTTAA TTATGATCAG 1260

AAGTCTCAGG AAGTTGAAGA CAAAACAAAG GAATATGAAT TGCTTAGTGA TGAATTGAAT 1320

CAAAAATCTG CAACTTTAGC AAGTATTGAT GCTGAGCTTC AGAAGCTGAA GGAAATGACC 1380

AACCACCAGA AGAAACGAGC AGCTGAAATG ATGGCATCAT TATTAAAAGA CCTTGCAGAA 1440

ATAGGAATTG CTGTGGGGAA TAACGATGTG AAGCAACCAG AAGGAACTGG TATGATAGAT 1500

GAAGAGTTTA CTGTTGCAAG ACTCTACATT AGCAAAATGA AATCAGAAGT AAAGACCATG 1560

GTGAAACGCT GCAAACAGCT AGAAAGCACG CAGACTGAGA GCAACAAAAA AATGGAAGAA 1620

AATGAGAAAG AGTTAGCAGC ATGCCAGCTT CGGATCTCCC AACATGAAGC CAAAATCAAG 1680

TCACTGACTG AGTACCTTCA GAATGTAGAA CAAAAGAAGA GGCAGCTGGA GGAATCTGTT 1740

GATTCCCTTG GTGAGGAGCT AGTCCAACTC CGAGCACAAG AGAAAGTCCA TGAAATGGAA 1800

AAAGAGCACT TGAACAAGGT TCAGACTGCA AATGAAGTCA AGCAAGCTGT TGAGCAGCAG 1860

ATCCAGAGTC ACAGAGAAAC CCACCAAAAA CAAATCAGTA GCTTGCGAGA TGAAGTTGAG 1920

GCAAAGGAAA AGCTAATCAC TGACCTCCAA GACCAAAACC AGAAGATGGT GTTGGAGCAG 1980

GAACGGCTAA GGGTGGAGCA TGAGAGGCTG AAGGCTACAG ACCAAGAGAA GAGCAGGAAG 2040

CTGCATGAGC TCACGGTTAT GCAAGACAGA CGAGAACAAG CAAGACAAGA CTTGAAGGGT 2100

TTGGAGGAGA CCGTGGCAAA AGAACTTCAG ACTTTACACA ACCTGCGTAA GCTCTTTGTT 2160

CAGGACTTGG CTACCAGGGT GAAAAAGAGG CCGAGGTCGA CTCTGACGAC ACTGGCGGCA 2220

GTGCTGCACA GAAGCAGAAA ATCTCCTTCC TTGAAAACAA CCTTGAACAG CTCACCAAAG 2280

TGCACAAGCA GTTGGTACGT GATAATGCAG ATCTTCGCTG TGAGCTTCCT AAGTTAGAGA 2340

AACGGCTTAG AGCTACTGCA GAAAGAGTGA AAGCTTTGGA GTCAGCCCG 2389

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

AGTGGCTGGA AAACGAGCTA 20

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CTTGATCCCT TCTGGTTGAT 20

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

AGTGGCTTGA AAATGAGCTC 20

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CTTGATCCCT TCTGGTAGAT G 21

WE CLAIM:

1. A method of identifying genetic suppressor elements that confer upon a cell resistance to one or more chemotherapeutic drug, the method comprising the steps of:

(a) synthesizing randomly fragmented cDNA prepared from the total mRNA of a cell to yield DNA fragments;

(b) transferring the DNA fragments to an expression vector to yield a library, wherein the expression vector is capable of expressing the DNA fragments in a living cell that is susceptible to inhibitory effects of a chemotherapeutic drug;

(c) genetically modifying living cells by introducing the genetic suppressor element library into the living cells;

(d) isolating or enriching for genetically modified living cells containing chemotherapeutic drug resistance-conferring genetic suppressor elements by selecting cells in the presence of a chemotherapeutic drug, and;

(e) obtaining the genetic suppressor element from the genetically modified cells.

2. A genetic suppressor element first identified by the method of claim 1.

3. A method according to claim 1, wherein the genetic suppressor element is a sense oriented genetic suppressor element encoding a peptide.

4. A method according to claim 1, wherein the genetic suppressor element is an antisense-oriented genetic suppressor element encoding an antisense RNA.

5. A synthetic peptide having an amino acid sequence corresponding to from about 6 amino acids to all of the amino acid sequence encoded by the GSE produced according to the method of claim 3.

6. A synthetic oligonucleotide having a nucleotide sequence from about 12 nucleotides to all of the nucleotide sequence of the antisense RNA encoded by the GSE produced by claim 4.

7. A method of identifying GSEs corresponding to genes that when suppressed by GSEs, confer a chemotherapeutic drug resistant selectable phenotype upon a cell having such suppressed genes, the method comprising the steps of:

(a) obtaining a total mRNA population from the cells;

(b) synthesizing randomly fragmented cDNA from the total mRNA;

(c) ligating the random cDNA fragments to synthetic adaptors to produce amplifiable random cDNA fragments;

(d) amplifying the random cDNA fragments;

(e) cloning the amplified mixture of random cDNA fragments into a suitable expression vector to produce a random fragment expression library;

(f) transferring the random fragment expression library into appropriate target cells;

(g) isolating or enriching for genetically modified living cells containing chemotherapeutic drug resistance-conferring genetic suppressor elements by selecting cells in the presence of a chemotherapeutic drug, and;

(h) recovering the GSE from the target cell having the selectable chemotherapeutic drug resistance phenotype.

8. A method for identifying GSEs corresponding to genes that, when suppressed by GSE, confer a selectable chemotherapeutic drug resistance phenotype upon a cell having such suppressed genes, the method comprising the steps of:

(a) obtaining genomic DNA from the cells;

(b) randomly fragmenting the genomic DNA to produce random genomic DNA fragments;

(c) ligating the random genomic DNA fragments to synthetic adaptors to produce amplifiable random genomic DNA fragments;

(d) amplifying the random genomic DNA fragments;

(e) cloning the amplified mixture of random genomic DNA fragments into a suitable expression vector to produce a random fragment expression library;

(f) transferring the random fragment expression library into appropriate target cells;

(g) isolating or enriching for genetically modified living cells containing chemotherapeutic drug resistance-conferring genetic suppressor elements by selecting cells in the presence of a chemotherapeutic drug; and

(h) recovering the GSE from the target cell having the selectable or screenable phenotype.

9. A synthetic peptide having an amino acid sequence that corresponds to from about 6 amino acids to all of an amino acid sequence of a peptide encoded by a GSE produced by the method of claim 7.

10. A synthetic oligonucleotide having a nucleotide sequence that corresponds to from about 12 nucleotides to all of the nucleotide sequence of an antisense RNA encoded by a GSE produced by the method of claim 7.

11. A synthetic peptide having an amino acid sequence that corresponds to from about 6 amino acids to all of the amino acid sequence of a peptide encoded by a GSE produced by the method of claim 8.

12. A synthetic oligonucleotide having a nucleotide sequence that corresponds to from about 12 nucleotides to all of the nucleotide sequence of an antisense RNA encoded by a GSE produced by the method of claim 8.

13. A genetic suppressor element first identified by the method of claim 7.

14. The genetic suppressor element according to claim 13, wherein the genetic suppressor element is a sense-oriented genetic suppressor element encoding a peptide.

15. The genetic suppressor element according to claim 13, wherein the genetic suppressor element is an antisense-oriented genetic suppressor element encoding an antisense RNA.

16. A genetic suppressor element first identified by the method of claim 8.

17. The genetic suppressor element according to claim 16, wherein the genetic suppressor element is a sense-oriented genetic suppressor element encoding a peptide.

18. The genetic suppressor element according to claim 16, wherein the genetic suppressor element is an antisense-oriented genetic suppressor element encoding an antisense RNA.

19. A method of isolating a gene associated with sensitivity to chemotherapeutic drugs, comprising the step of screening a cDNA library with an oligonucleotide or polynucleotide having a nucleotide sequence of from about 12 to all of the nucleotides of the GSE according to claim 2.

20. A method of isolating a gene associated with sensitivity to chemotherapeutic drugs, comprising the step of screening a cDNA library with an oligonucleotide or polynucleotide having a nucleotide sequence of from about 12 to all of the nucleotides of the GSE according to claim 13.

21. A method of isolating a gene associated with sensitivity to chemotherapeutic drugs, comprising the step of screening a cDNA library with an oligonucleotide or polynucleotide having a nucleotide sequence of from about 12 to all of the nucleotides of the GSE according to claim 16.

22. A method of isolating a gene associated with sensitivity to chemotherapeutic drugs, comprising the step of screening a genomic library with an oligonucleotide or polynucleotide having a nucleotide sequence of from about 12 to all of the nucleotides of the GSE according to claim 2.

23. A method of isolating a gene associated with sensitivity to chemotherapeutic drugs, comprising the step of screening a genomic library with a oligonucleotide or polynucleotide having a nucleotide sequence of from about 12 to all of the nucleotides of the GSE according to claim 13.

24. A method of isolating a gene associated with sensitivity to chemotherapeutic drugs, comprising the step of screening a genomic library with

an oligonucleotide or polynucleotide having a nucleotide sequence of from about 12 to all of the nucleotides of the GSE according to claim 16.

25. A cloned gene first isolated by the method of claim 19.

26. A cloned gene first isolated by the method of claim 20.

27. A cloned gene first isolated by the method of claim 21.

28. A cloned gene first isolated by the method of claim 22.

29. A cloned gene first isolated by the method of claim 23.

30. A cloned gene first isolated by the method of claim 24.

31. A diagnostic assay for chemotherapeutic drug resistance comprising the step of quantitating the gene expression of a gene comprising a nucleotide sequence homologous to that of the GSE according to claim 2.

32. A diagnostic assay for chemotherapeutic drug resistance comprising the step of quantitating the gene expression of a gene comprising a nucleotide sequence homologous to that of the GSE according to claim 13.

33. A diagnostic assay for chemotherapeutic drug resistance comprising the step of quantitating the gene expression of a gene comprising a nucleotide sequence homologous to that of the GSE according to claim 16.

34. A genetic suppressor element according to claim 2 having a nucleotide sequence that is homologous to a portion of the human or mouse *khcs* gene.

35. A genetic suppressor element according to claim 2 having a nucleotide sequence that is homologous to a portion of a gene comprising the nucleotide sequence shown in Figure 10.

36. A genetic suppressor element according to claim 2 having a nucleotide sequence that is homologous to a portion of a gene comprising the nucleotide sequence shown in Figure 11.

37. A genetic suppressor element according to claim 2 having a nucleotide sequence that is homologous to the nucleotide sequence or its complement shown in Figure 9.

38. A genetic suppressor element according to claim 2 having a nucleotide sequence that is homologous to the nucleotide sequence shown in Figure 10 or its complement.

39. A genetic suppressor element according to claim 2 having a nucleotide sequence that is homologous to the nucleotide sequence shown in Figure 11 or its complement.

40. A genetic suppressor element according to claim 13 having a nucleotide sequence that is homologous to a portion of the human or mouse *khcs* gene.

41. A genetic suppressor element according to claim 13 having a nucleotide sequence that is homologous to a portion of a gene comprising the nucleotide sequence shown in Figure 10.

42. A genetic suppressor element according to claim 13 having a nucleotide sequence that is homologous to a portion of a gene comprising the nucleotide sequence shown in Figure 11.

43. A genetic suppressor element according to claim 13 having a nucleotide sequence that is homologous to the nucleotide sequence shown in Figure 9 or its complement.

44. A genetic suppressor element according to claim 13 having a nucleotide sequence that is homologous to the nucleotide sequence shown in Figure 10 or its complement.

45. A genetic suppressor element according to claim 13 having a nucleotide sequence that is homologous to the nucleotide sequence shown in Figure 11 or its complement.

46. A genetic suppressor element according to claim 16 having a nucleotide sequence that is homologous to a portion of the human or mouse *khcs* gene.

47. A genetic suppressor element according to claim 16 having a nucleotide sequence that is homologous to a portion of a gene comprising the nucleotide sequence as shown in Figure 10.

48. A genetic suppressor element according to claim 16 having a nucleotide sequence that is homologous to a portion of a gene comprising the nucleotide sequence as shown in Figure 11.

49. A genetic suppressor element according to claim 16 having a nucleotide sequence that is homologous to the nucleotide sequence or its complement shown in Figure 9.

50. A genetic suppressor element according to claim 16 having a nucleotide sequence that is homologous to the nucleotide sequence shown in Figure 10 or its complement.

51. A genetic suppressor element according to claim 16 having a nucleotide sequence that is homologous to the nucleotide sequence shown in Figure 11 or its complement.

52. A mammalian cell that expresses a GSE according to claim 2.

53. A mammalian cell that expresses a GSE according to claim 13.

54. A mammalian cell that expresses a GSE according to claim 16.

55. A mammalian cell that expresses a GSE according to claim 34.

56. A mammalian cell that expresses a GSE according to claim 35.

57. A mammalian cell that expresses a GSE according to claim 36.

58. A mammalian cell that expresses a GSE according to claim 37.

59. A mammalian cell that expresses a GSE according to claim 38.

60. A mammalian cell that expresses a GSE according to claim 39.

61. An antibody raised against a protein encoded by, or a peptide encoded by a portion of, a gene to which a GSE according to claim 2 is homologous.

62. An antibody raised against a protein encoded by, or a peptide encoded by a portion of, a gene to which a GSE according to claim 13 is homologous.

63. An antibody raised against a protein encoded by, or a peptide encoded by a portion of, a gene to which a GSE according to claim 16 is homologous.

64. An antibody raised against a protein encoded by, or a peptide encoded by a portion of, a gene to which a GSE according to claim 34 is homologous.

65. An antibody raised against a protein encoded by, or a peptide encoded by a portion of, a gene to which a GSE according to claim 35 is homologous.

66. An antibody raised against a protein encoded by, or a peptide encoded by a portion of, a gene to which a GSE according to claim 36 is homologous.

67. A method of overcoming drug resistance in a cancer cell, comprising the step of expressing in the cancer cell a complete gene to which a GSE according to claim 2 is homologous.

68. A method of overcoming drug resistance in a cancer cell, comprising the step of expressing in the cancer cell a complete gene to which a GSE according to claim 13 is homologous.

69. A method of overcoming drug resistance in a cancer cell, comprising the step of expressing in the cancer cell a complete gene to which a GSE according to claim 16 is homologous.

70. A method of overcoming drug resistance in a cancer cell, comprising the step of expressing in the cancer cell a complete gene to which a GSE according to claim 34 is homologous.

71. A method of overcoming drug resistance in a cancer cell, comprising the step of expressing in the cancer cell a complete gene to which a GSE according to claim 35 is homologous.

72. A method of overcoming drug resistance in a cancer cell, comprising the step of expressing in the cancer cell a complete gene to which a GSE according to claim 36 is homologous.

73. A method of identifying genetic suppressor elements that confer upon a cell resistance to one or more chemotherapeutic drugs, the method comprising the steps of:

(a) obtaining random DNA fragments of a gene associated with sensitivity to chemotherapeutic drugs;

(b) transferring the DNA fragments to an expression vector to yield a library, wherein the expression vector is capable of expressing the DNA fragments in a living cell that is susceptible of inhibitory effects of a chemotherapeutic drug;

(c) genetically modifying living cells by introducing the genetic suppressor element library into the living cells;

(d) isolating or enriching for genetically modified living cells containing chemotherapeutic drug resistance-conferring genetic suppressor elements by selecting cells in the presence of chemotherapeutic drug, and;

(e) obtaining a genetic suppressor element from the genetically modified cells.

74. The method of claim 73, wherein the cloned gene has a nucleotide sequence to which the GSE shown in Figure 9 is homologous.

75. The method of claim 73, wherein the cloned gene has a nucleotide sequence to which the GSE shown in Figure 10 is homologous.

76. The method of claim 73, wherein the cloned gene has a nucleotide sequence to which the GSE shown in Figure 11 is homologous.

77. A GSE first identified by the method of claim 73.

78. A GSE first identified by the method of claim 74.

79. A GSE first identified by the method of claim 75.

80. A GSE first identified by the method of claim 76.

81. A mammalian cell that expresses a GSE according to claim 77.

82. A mammalian cell that expresses a GSE according to claim 78.

83. A mammalian cell that expresses a GSE according to claim 79.

84. A mammalian cell that expresses a GSE according to claim 80.

85. A synthetic oligonucleotide having a nucleotide sequence comprising from about 12 to all of the nucleotide sequence of a GSE according to claim 77.

86. A synthetic oligonucleotide having a nucleotide sequence comprising from about 12 to all of the nucleotide sequence of a GSE according to claim 78.

87. A synthetic oligonucleotide having a nucleotide sequence comprising from about 12 to all of the nucleotide sequence of a GSE according to claim 79.

88. A synthetic oligonucleotide having a nucleotide sequence comprising from about 12 to all of the nucleotide sequence of a GSE according to claim 80.

89. A synthetic peptide having an amino acid sequence comprising from about 6 to all of the amino acids encoded by a GSE according to claim 77.

90. A synthetic peptide having an amino acid sequence comprising from about 6 to all of the amino acids encoded by a GSE according to claim 78.

91. A synthetic peptide having an amino acid sequence comprising from about 6 to all of the amino acids encoded by a GSE according to claim 79.

92. A synthetic peptide having an amino acid sequence comprising from about 6 to all of the amino acids encoded by a GSE according to claim 80.

93. A GSE according to claim 77, having a nucleotide sequence selected from the group consisting of the nucleotide sequences shown in Figure 1.

Figure 1

A

```
GTGTCTGGGC GGAGCAAAAT ATGTTCCAAT TGTGTTTTCT TTTGATAGAT TCTTTGAACA   60
GACAGTCTTT TCTTAGCATC TTCATTTTTC TTTATTTTGT TGACTTGCAT ATTTTCATTT  120
ACAGGCTGCA ATGGTGACAC TTCCATGGTG ACGGTCGTGA AGGG                   164
```

B

```
TGAAAAGATG TATGTCCCAG CTCTCATATT TGGACAGCTC CTAACTTCTA GTAACTATGA   60
TGATGATGAA AAGAAAGTGA CAGGTGGTCG AAATGGCTAT GGAGCCAAAT TGTGTAACAT  120
ATTCAGTACC AAATTTACTG TGGAAACAGC CAGTAGAGAA TACAAGAAAA TGTTCAAACA  180
GACATGGATG GATAATATGG GAAGAGCTGG TGA                               213
```

C

```
GCCCATTGGT CAGTTTGGTA CCAGGCTACA TGGTGGCAAG GATTCTGCTA GTCCACGATA   60
CATCTTTACA ATGCTCAGCT CTTTGGCTCG ATTGTTATTT CCACCAAAAG ATGATCACAC  120
GTTGAAGTTT TTATATGATG ACAACCAGCG TGTTGAGCCT GAATGGTACA TTCCTATTAT  180
T                                                                  181
```

D

```
TGAATGGTAC ATTCCTATTA TTCCCATGGT GCTGATAAAT GGTGCTGAAG GAATCGGTAC   60
TGGGTGGTCC TGCAAAATCC CCAACTTTGA TGTGCGTGAA ATTGTAAATA ACATCAGGCG  120
TTTGATGGAT GGAGAAGAAC CTTTGCCAAT GCTTCCAAGT TACAAGAACT TCAAGGGTAC  180
TATTGAAGAA CTGGCTCCAA ATCAATATGT GATTAGTGGT GAAG                    224
```

Figure 1 (cont'd.)

E

```
TGCGTGAAAT TGTAAATAAC ATCAGGCGTT TGATGGATGG AGAAGAACCT TTGCCAATGC      60
TTCCAAGTTA CAAGAACTTC AAGGGTACTA TTGAAGAACT GGCTCCAAAT CAATATGTGA     120
TTAGTGGTGA AGTAGCTATT CTTAATTCTA CAACCATTGA AATCTCAGAG CTTCCCGTCA     180
GAACATGGAC CCAGACATAC AAAGAACAAG TTCTAGAACC CATGTTGAAT GGCACCGAGA     240
AGACACCTCC TCTCATAACA GACTATAGGG AATACCATAC AGATACCACT GTGAAATTTG     300
TTGTGAAGAT GACTGAAGAA AAACTGGCA                                       329
```

F

```
CACTCTTTTC AGTTTCCTTT TCGTTGTCAC TCTCTTCATT TTCTTCTTCA TCTGGAACCT      60
TTTGCTGGGC TTCTTTCCAG GCCTTCACAG GATCCGAATC ATATCCCCTC TGAATCAGAA     120
CTTTAATTAA TTCTTTCTTA GGCTTATTTT CAATGATTAT TTTGCCATCT ATTTTCTCTA     180
AGATAAAGCG AGCC                                                      194
```

G

```
TCTGCCTCTG CTTTCATTTC TATGGTTATT CGTGGAATGA CTCTTTGACC ACGCGGAGAA      60
GGCAAAACTT CAGCCATTTG TGTTTTTTTC CCCTTGGCCT TCCCCCCTTT CCCAGGAAGT     120
CCGACTTGTT CATCTTGTTT TTCCTTGGCT TCAACAGCCT CCAATTCTTC AATAAATGTA     180
GCCAAGTCTT CTTTCCACAA ATCTGA                                          206
```

H

```
GACACGACAC TTTTCTGTGG TTTCAGTTCT TTGTTACTAA GTTTTGGGGA AGTTTTGGTC      60
TTAGGTGGAC TAGCATCTGA TGGGACAAAA TCTTCATCAT CAGTTTTTTC ATCAAAATCT     120
GAGAAATCTT CATCTGAATC CAAATCCATT GTGAATTTTG TTTTTGTTGC TGCTCTCCGT     180
GGCTCTGTTT CTCG                                                      194
```

Figure 1 (cont'd.)

I

```
CTGAAACCAC AGAAAAGTGT CGTGTCAGAC CTTGAAGCTG ATGATGTTAA GGGCAGTGTA    60
CCACTGTCTT CAAGCCCTCC TGCTACACAT TTCCCAGATG AAACTGAAAT TACAAACCCA   120
GTTCCTAAAA AGAATGTGAC AGTGAAGAAG ACAGCAGCAA AAAGTCAGTC TTCCACCTCC   180
ACTACCGGTG CCAAAAAAAG GGCTGCCCCA AAAGGAACTA AAAGGGATCC AGCTTTGAAT   240
TC                                                                  242
```

J

```
AATTCAAAGC TGGATCCCTT TTAGTTCCTT TTGGGGCAGC CCTTTTTTTG GCACCGGTAG    60
TGGAGGTGGA AGACTGACTT TTTGCTGCTG TCTTCTTCAC TGTCACATTC TTTTTAGGAA   120
CTGGGTTTGT AATTTCAGTT TCATCTGGGA AATGTGTAGC AGGAGGGCTT GAAGACAGTG   180
GTACACTGCC CTTAACATCA TCAGCTTCAA GGTCTGACAC                          220
```

Figure 2A

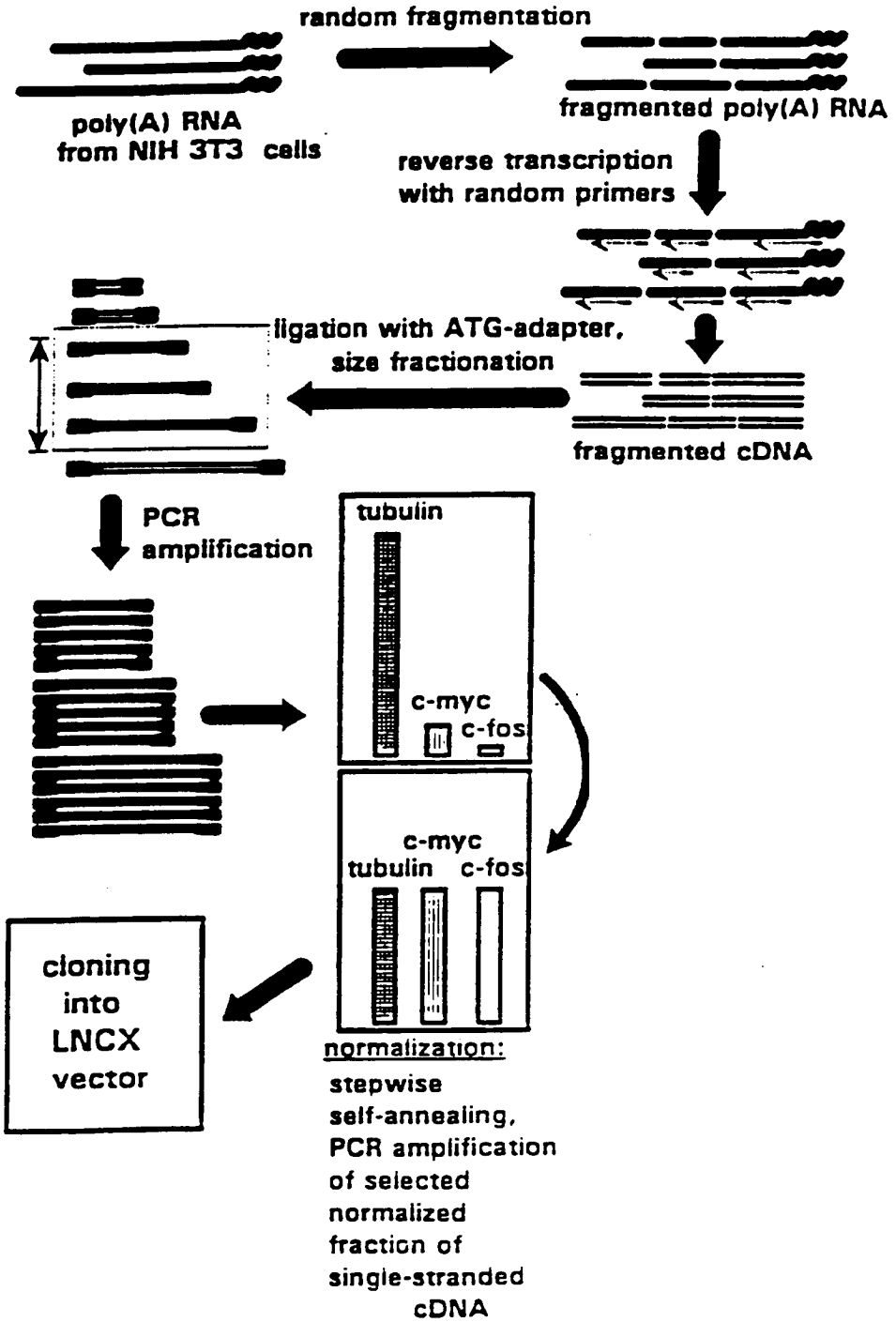

random fragmentation
poly(A) RNA
from NIH 3T3 cells
fragmented poly(A) RNA
reverse transcription
with random primers
ligation with ATG-adapter,
size fractionation
fragmented cDNA
PCR
amplification
tubulin
c-myc
c-fos
c-myc
tubulin
c-fos
cloning
into
LNCX
vector
normalization:
stepwise
self-annealing,
PCR amplification
of selected
normalized
fraction of
single-stranded
cDNA

Figure 2B

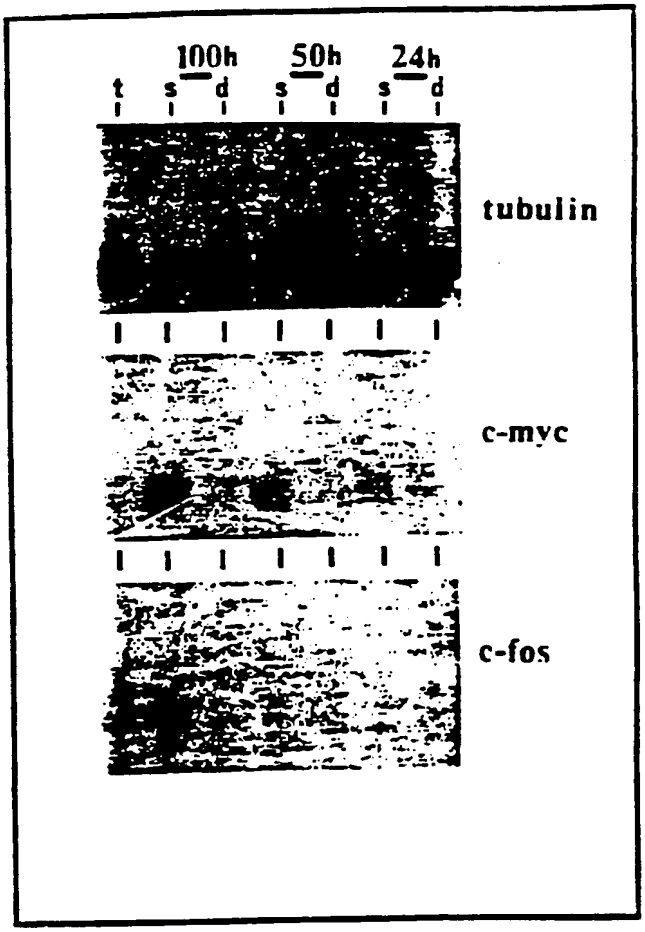


Figure 3

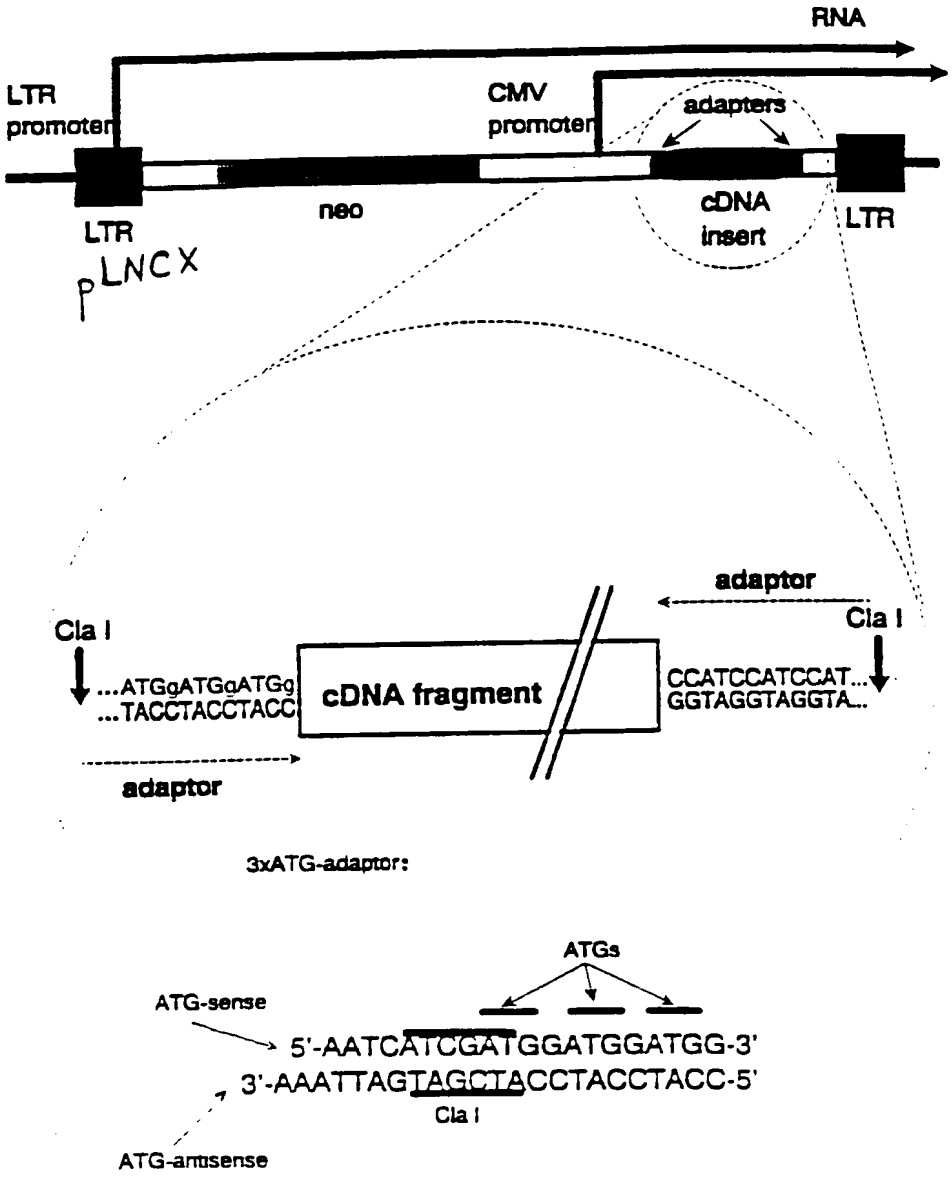

RNA
LTR promoter
CMV promoter
adapters
neo
cDNA insert
LTR
LTR
pLNCX
adaptor
Cla I
Cla I
...ATGgATGgATGg
...TACCTACCTACC
cDNA fragment
CCATCCATCCAT...
GGTAGGTAGGTA...
adaptor
3xATG-adaptor:
ATGs
ATG-sense
5'-AATCATCGATGGATGGATGG-3'
3'-AAATTAGTAGCTACCTACCTACC-5'
Cla I
ATG-antisense

Figure 4

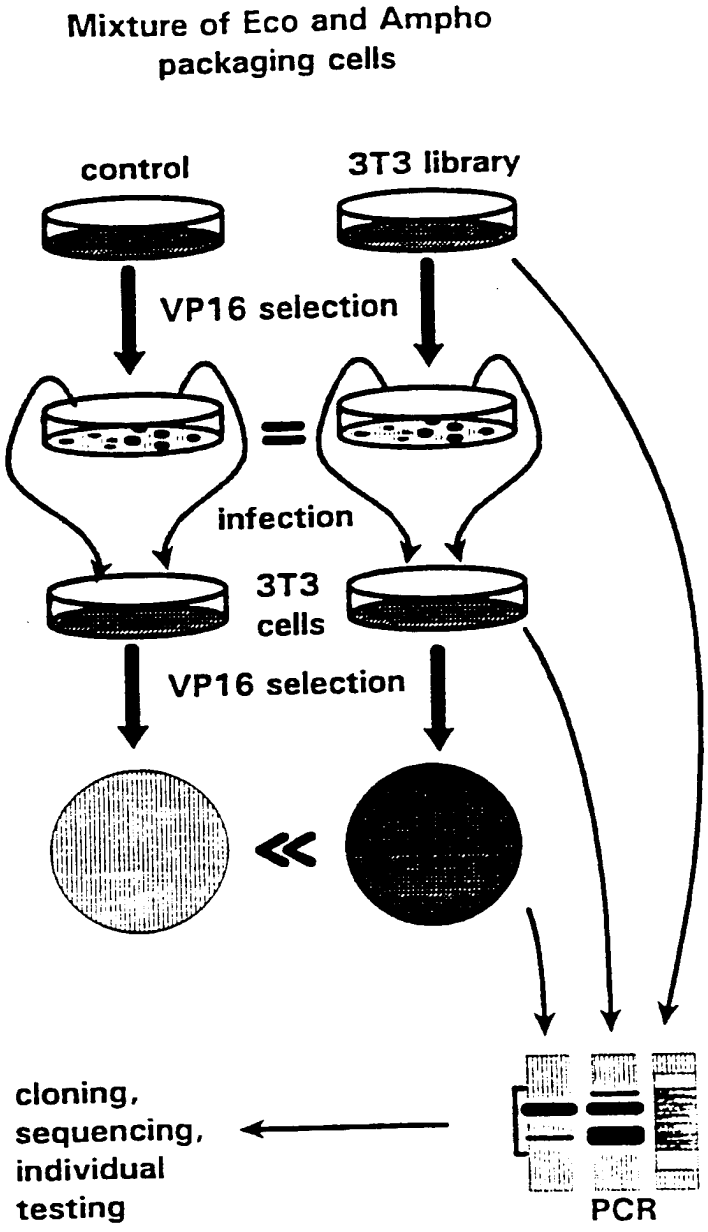
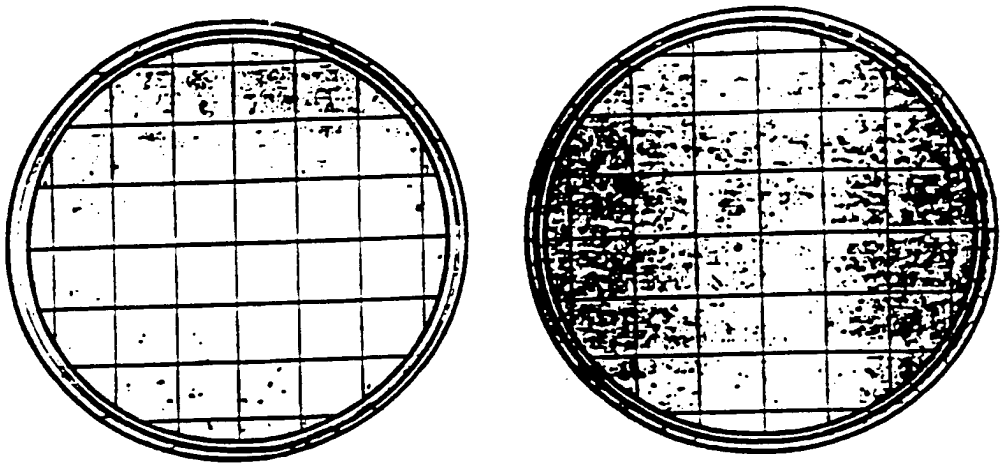

Mixture of Eco and Ampho packaging cells
control
3T3 library
VP16 selection
infection
3T3 cells
VP16 selection
cloning, sequencing, individual testing
PCR

Figure 5A

VP16 selection of 3T3 cells infected by virus from preselected packaging cells



control

infection

Figure 5B

PCR amplification of inserts from 3T3 cells infected by normalized 3T3 cDNA library

VP16 selection:

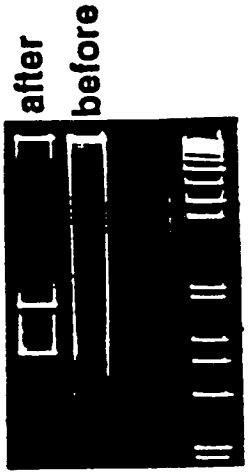


Figure 6

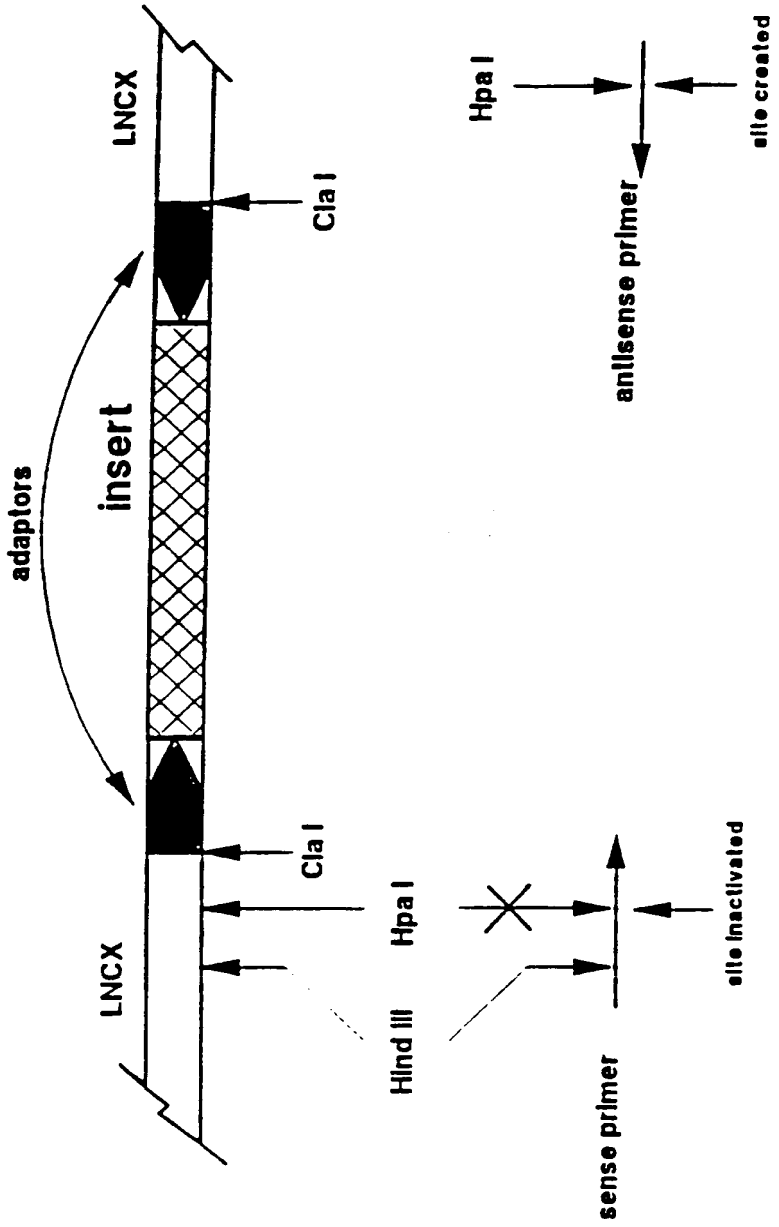

LNCX
Cla I
adaptors
insert
Cla I
LNCX
Hpa I
Hind III
Hpa I
site created
antisense primer
site inactivated
sense primer

Figure 7A

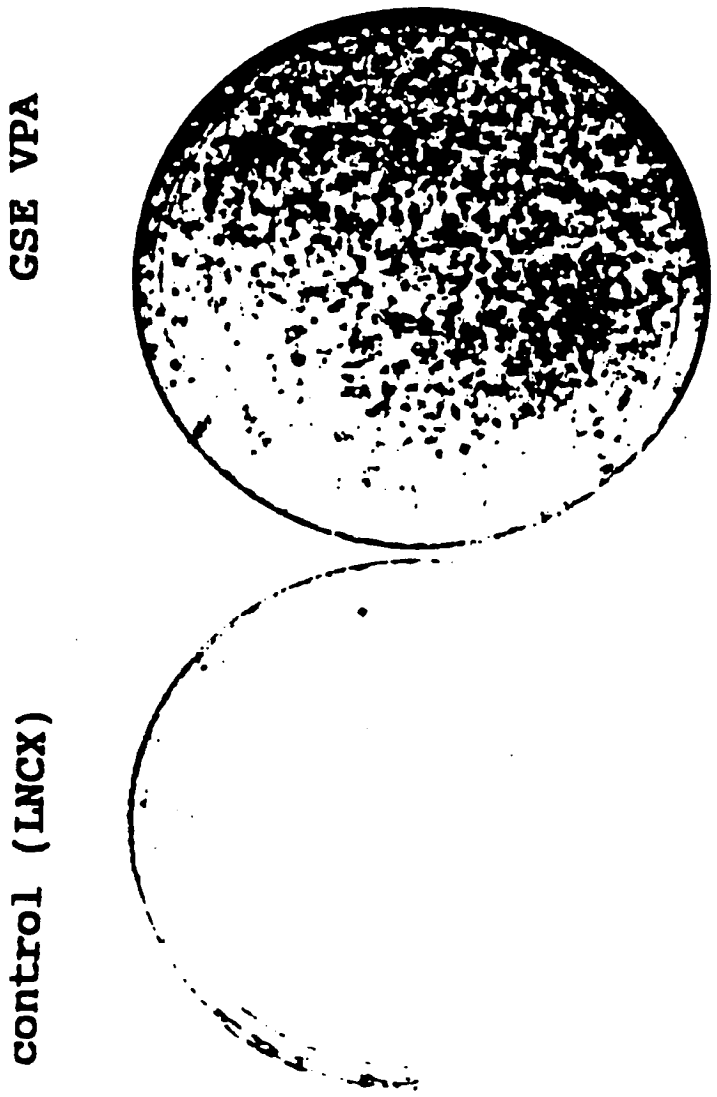


Figure 7B

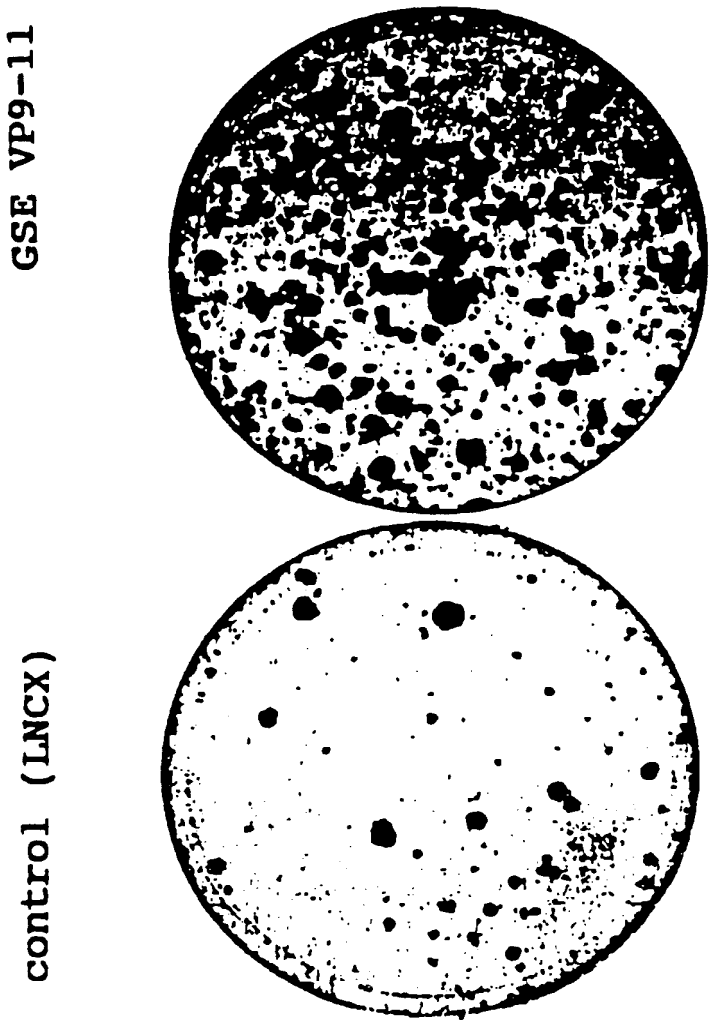


Figure 8A

Biological effects of khcs GSE isolated from a normalized NIH 3T3 cDNA RFRL

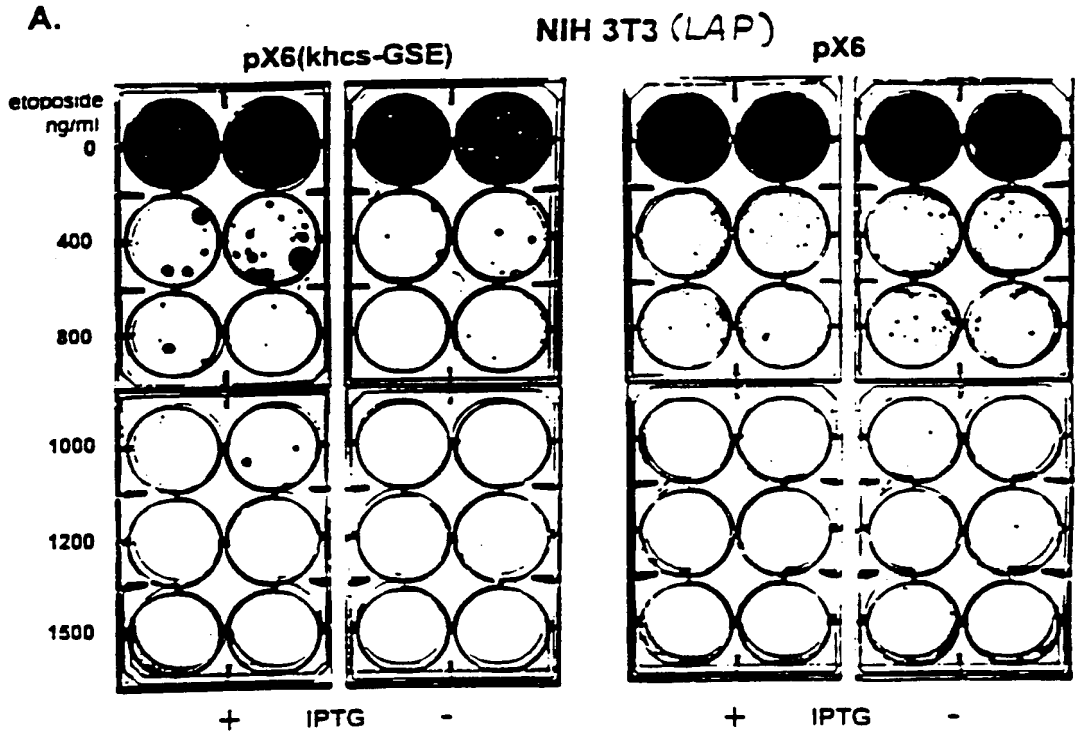


Figure 8B

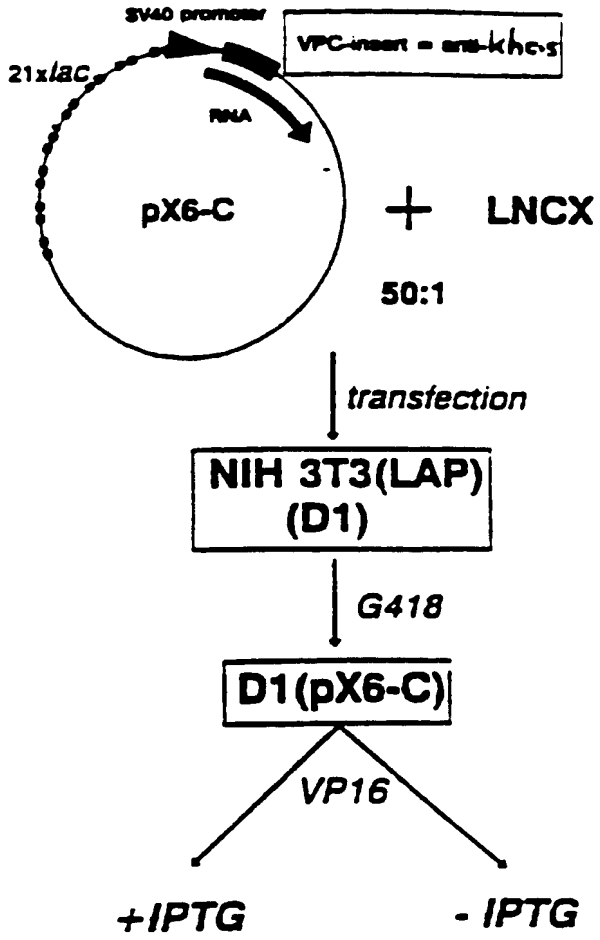

SV40 promoter
VPC-insert = ets-khc-s
21xlac
RNA
pX6-C
+
LNCX
50:1
transfection
NIH 3T3(LAP)
(D1)
G418
D1(pX6-C)
VP16
+IPTG
- IPTG

Figure 9

```
GSE VPC (anti khc-s)
CTTGATCCCT TCTGGTTGAT GCCAGAAGCT CTTCCTGATC CAGCATTTGT ATCTTCAATT   60
TCTCTACCAA TTGGCTTTGT TGGTTAATCT CTTCATCCTT GTCATCAAGT TGTTTATACA  120
ATTTAGCAAG TTCTTCTTCA CACTTTCTTC TTTCAGCATC GGTAAAACTA CCAGCCATTC  180
CGACTGCAGC AGCTGGTTTA TCACTGGTAA TAGCAATATC TTTATCCGCT GTGAAGGCTT  240
CCAAATTAGC TTTCTCTTTG TCAAACTGCT CATCAATAGG CACTGTCTCC CCGTTACGCC  300
AACGGTTTAG CTCGTTTTCC AGCCACT                                      327
```

Figure 10

```
GSE VPA
CCGACCGGGA GCGGGAGAAG GAGCGGGAGC GGGAGCAGCG GGAGAAGGAG CGGGAGAAGG   60
AGCTGGAGCG CGACGGGAGA AGGAACGGGA GCGCGAGCTG GAGCGGCAGC GGGAGCAGCG  120
GGCGAGGGAG AAGGAGCTGC TGGCTGCCAA GGCCTTAGAG CCCACCACCT TCCTGCCTGT  180
GGCCGAGCTG CACGGACTCC GAGGTCACAG CACGGAGGAG CGGCCCAAGC CCTCGGAGCA  240
GCTGACCCCA                                                         250
```

Figure 11

```
GSE VP9-11
CTCAGAGGTG ATCCTCTCGG AGTCGAGCTC AGGAGAAGGA GTCCCCTTCT TTGAGACTTG   60
GATGCAGACC TGCATGTCCG AGGAGGGCAA GATTTTGAAC CCTGACCATC CCTGCTTCCG  120
CCCTGACTCC ACCGAAGTCG AGTCCTTGGT GGCCCTGCTC AACAACTCTT CAGAGATGAA  180
GCTAGTACAG ATGAAGTAGC ACGAGGCC                                     208
```

Figure 12

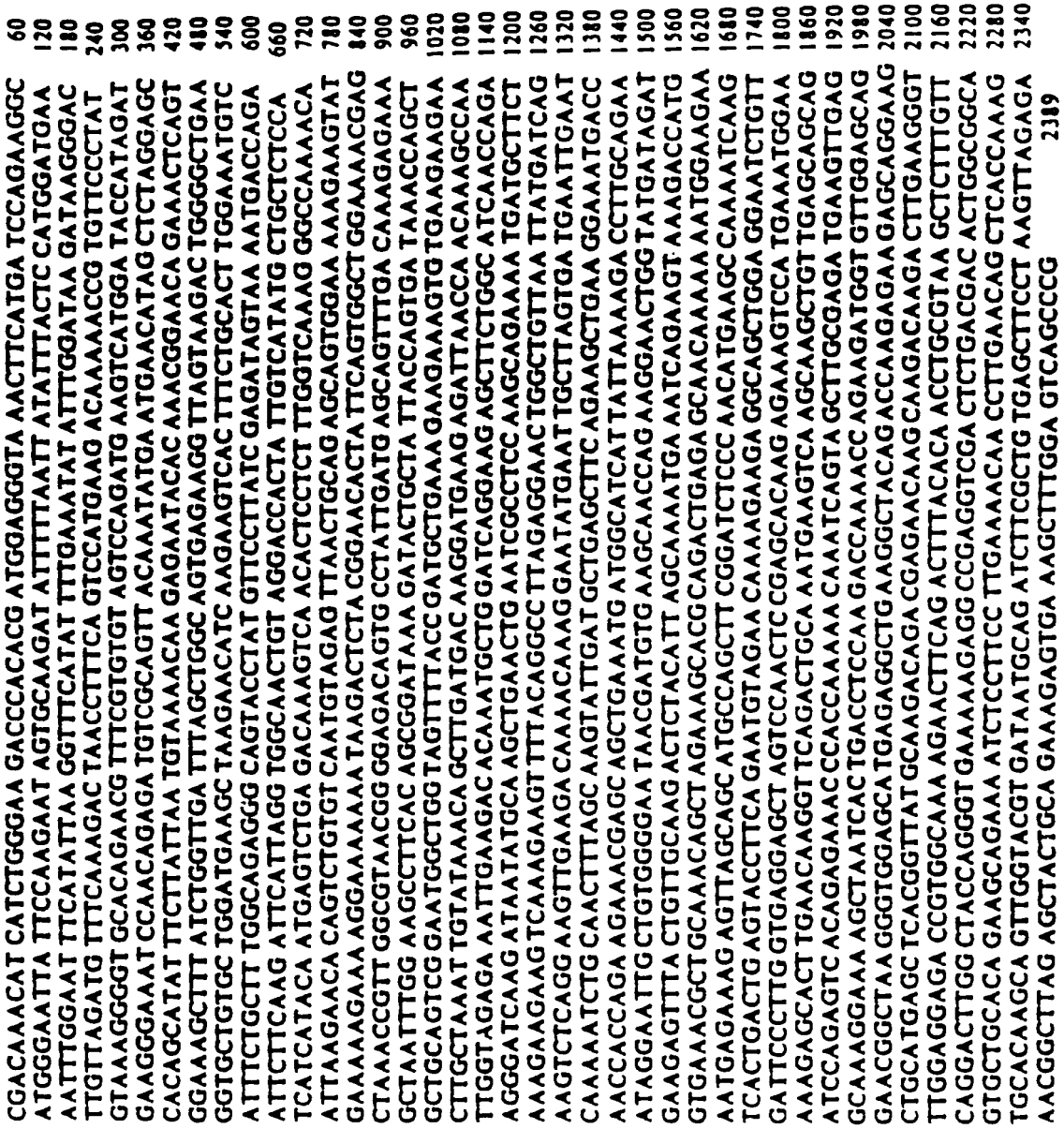

CGACAAACAT CATCTGGGAA GACCCACACG ATGGAGGGTA AACTTCATGA TCCAGAAGGC 60
ATGGGAATTA TTCCAAGAAT AGTGCAAGAT ATTTTTAATT ATATTTACTC CATGGATGAA 120
AATTTGGAAT TTCATATTAA GGTTTCATAT TTTGAAATAT ATTTGGATAA GATAAGGGAC 180
TTGTTAGATG TTTCAAAGAC TAACCTTTCA GTCCATGAAG ACAAAAACCG TGTTCCCTAT 240
GTAAAGGGGT GCACAGAACG TTTCGTGTGT AGTCCAGATG AAGTCATGGA TACCATAGAT 300
GAAGGGAAAT CCAACAGAGA TGTCGCAGTT ACAAATATGA ATGAACATAG CTCTAGGAGC 360
CACAGCATAT TTCTTATTAA TGTAAAACAA GAGAATACAC AAACGGAACA GAAACTCAGT 420
GGAAAGCTTT ATCTGGTTGA TTTAGCTGGC AGTGAGAAGG TTAGTAAGAC TGGGGCTGAA 480
GGTGCTGTGC TGGATGAAGC TAAGAACATC AAGAAGTCAC TTTCTGCACT TGGAAATGTC 540
ATTTCTGCTT TGGCAGAGGG CAGTACCTAT GTTCCTTATC GAGATAGTAA AATGACCAGA 600
ATTCTTCAAG ATTCATTAGG TGGCAACTGT AGGACCACTA TTGTCATATG CTGCTCTCCA 660
TCATCATACA ATGAGTCTGA GACAAAGTCA ACACTCCTCT TTGGTCAAAG GGCCAAAACA 720
ATTAAGAACA CAGTCTGTGT CAATGTAGAG TTAACTGCAG AGCAGTGGAA AAAGAAGTAT 780
GAAAAAGAAA AGGAAAAAAA TAAGACTCTA CGGAACACTA TTCAGTGGCT GGAAAACGAG 840
CTAAACCGTT GGCGTAACGG GGAGACAGTG CCTATTGATG AGCAGTTTGA CAAAGAGAAA 900
GCTAATTTGG AAGCCTTCAC AGCGGATAAA GATACTGCTA TTACCAGTGA TAAACCAGCT 960
GCTGCAGTCG GAATGGCTGG TAGTTTTACC GATGCTGAAA GAAGAAAGTG TGAAGAAGAA 1020
CTTGCTAAAT TGTATAAACA GCTTGATGAC AAGGATGAAG AGATTAACCA ACAAAGCCAA 1080
TTGGTAGAGA AATTGAAGAC ACAAATGCTG GATCAGGAAG AGCTTCTGGC ATCAACCAGA 1140
AGGGATCAAG ATAATATGCA AGCTGAACTG AATCGCCTCC AAGCAGAAAA TGATGCTTCT 1200
AAAGAAGAAG TCAAAGAAGT TTTACAGGCC TTAGAGGAACTGGCTGTTAA TTATGATCAG 1260
AAGTCTCAGG AAGTTGAAGA CAAAACAAAG GAATATGAAT TGCTTAGTGA TGAATTGAAT 1320
CAAAAATCTG CAACTTTAGC AAGTATTGAT GCTGAGCTTC AGAAGCTGAA GGAAATGACC 1380
AACCACCAGA AGAAACGAGC AGCTGAAATG ATGGCATCAT TATTAAAAGA CCTTGCAGAA 1440
ATAGGAATTG CTGTGGGGAA TAACGATGTG AAGCAACCAG AAGGAACTGG TATGATAGAT 1500
GAAGAGTTTA CTGTTGCAAG ACTCTACATT AGCAAAATGA AATCAGAAGT AAAGACCATG 1560
GTGAAACGCT GCAAACAGCT AGAAAGCACG CAGACTGAGA GCAACAAAAA AATGGAAGAA 1620
AATGAGAAAG AGTTAGCAGC ATGCCAGCTT CGGATCTCCC AACATGAAGC CAAAATCAAG 1680
TCACTGACTG AGTACCTTCA GAATGTAGAA CAAAAGAAGA GGCAGCTGGA GGAATCTGTT 1740
GATTCCCTTG GTGAGGAGCT AGTCCAACTC CGAGCACAAG AGAAAGTCCA TGAAATGGAA 1800
AAAGAGCACT TGAACAAGGT TCAGACTGCA AATGAAGTCA AGCAAGCTGT TGAGCAGCAG 1860
ATCCAGAGTC ACAGAGAAAC CCACCAAAAA CAAATCAGTA GCTTGCGAGA TGAAGTTGAG 1920
GCAAAGGAAA AGCTAATCAC TGACCTCCAA GACCAAAACC AGAAGATGGT GTTGGAGCAG 1980
GAACGGCTAA GGGTGGAGCA TGAGAGGCTG AAGGCTACAG ACCAAGAGAA GAGCAGGAAG 2040
CTGCATGAGC TCACGGTTAT GCAAGACAGA CGAGAACAAG CAAGACAAGA CTTGAAGGGT 2100
TTGGAGGAGA CCGTGGCAAA AGAACTTCAG ACTTTACACA ACCTGCGTAA GCTCTTTGTT 2160
CAGGACTTGG CTACCAGGGT GAAAAAGAGG CCGAGGTCGA CTCTGACGAC ACTGGCGCA 2220
GTGCTGCACA GAAGCAGAAA ATCTCCTTCC TTGAAAACAA CCTTGAACAG CTCACCAAAG 2280
TGCACAAGCA GTTGGTACGT GATAATGCAG ATCTTCGCTG TGAGCTTCCT AAGTTAGAGA 2340
AACGGCTTAG AGCTACTGCA GAAAGAGTGA AAGCTTTGGA GTCAGCCCG 2389

Figure 13A

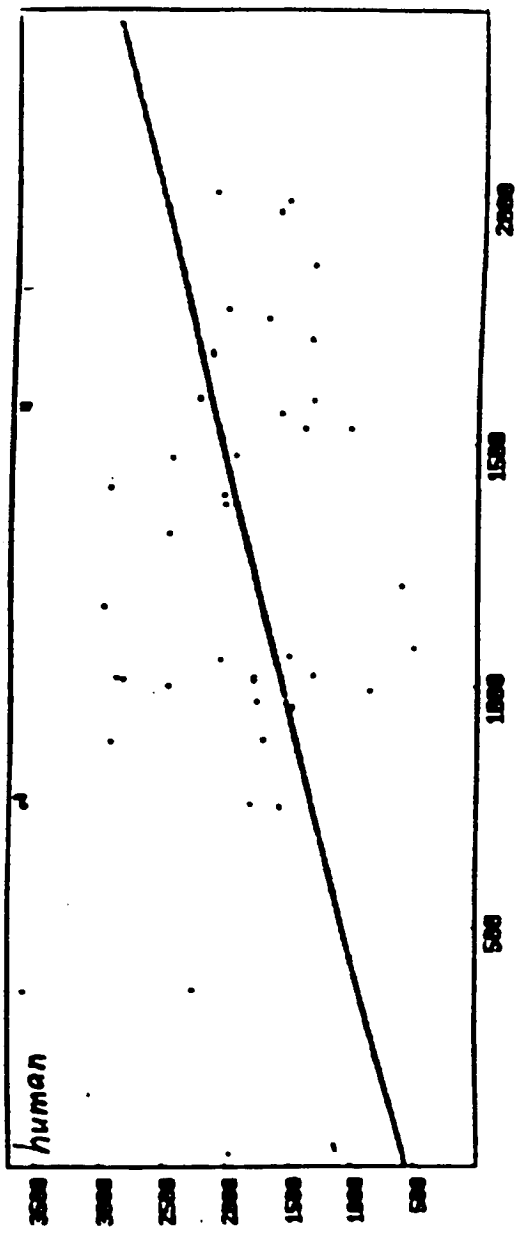


Figure 13B

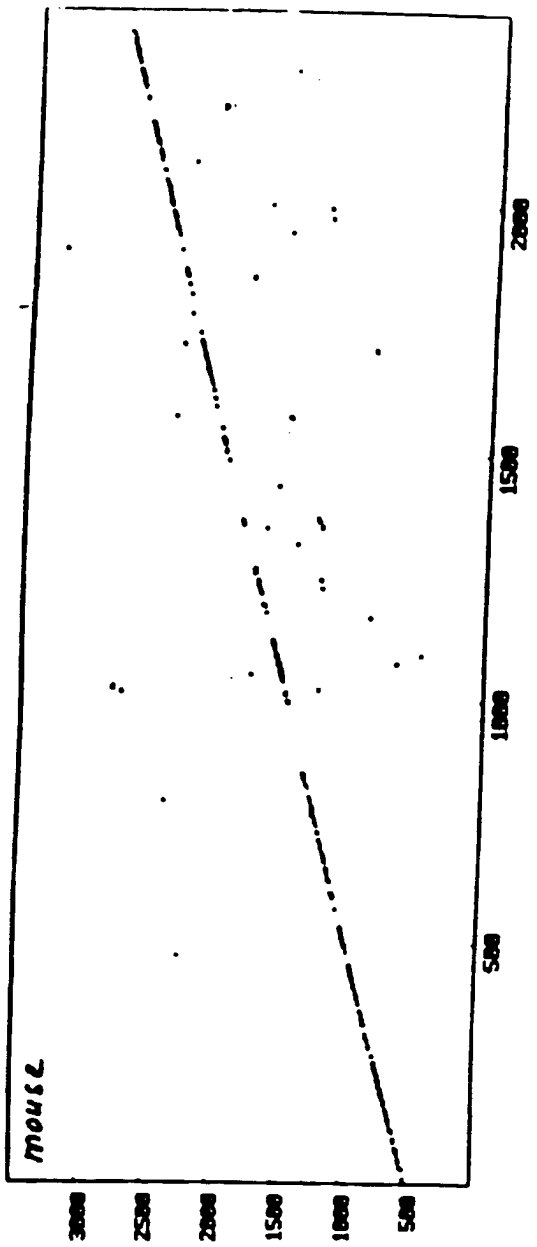


Figure 13C

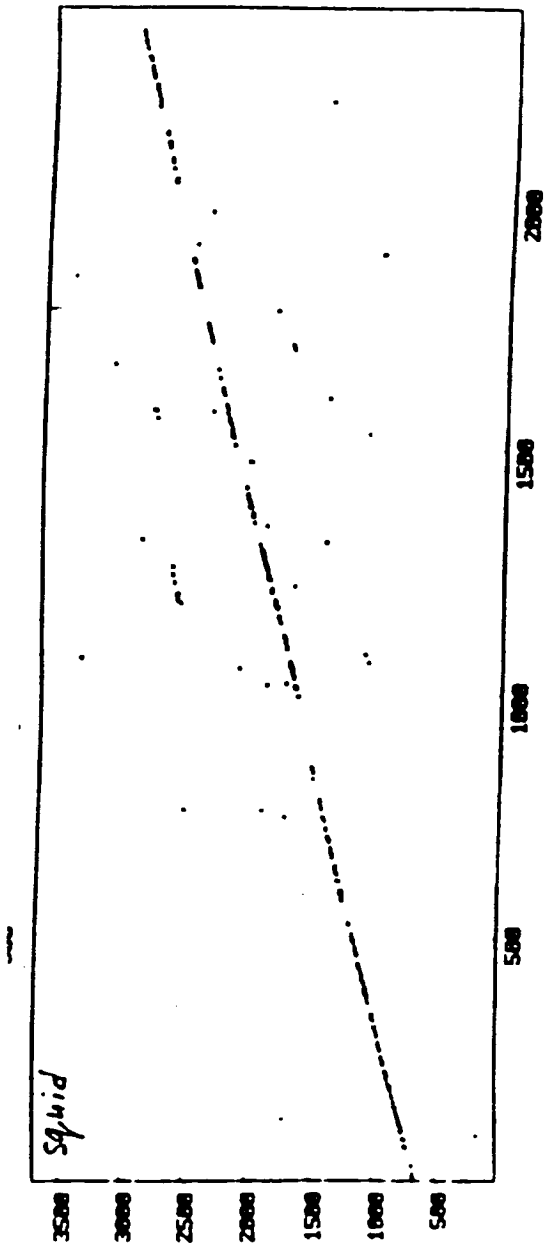


Figure 13D

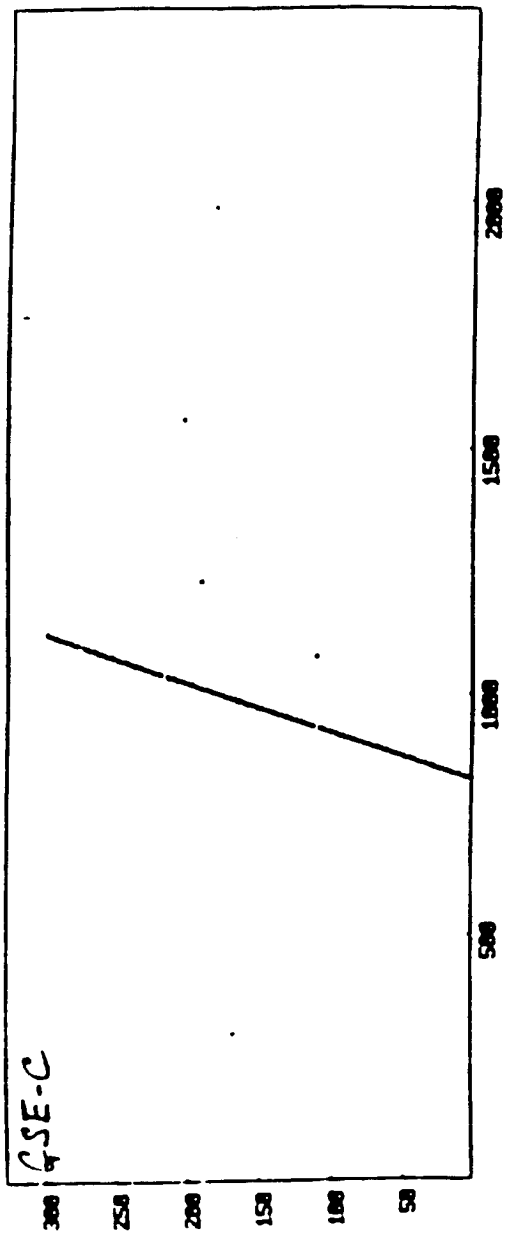


Figure 14

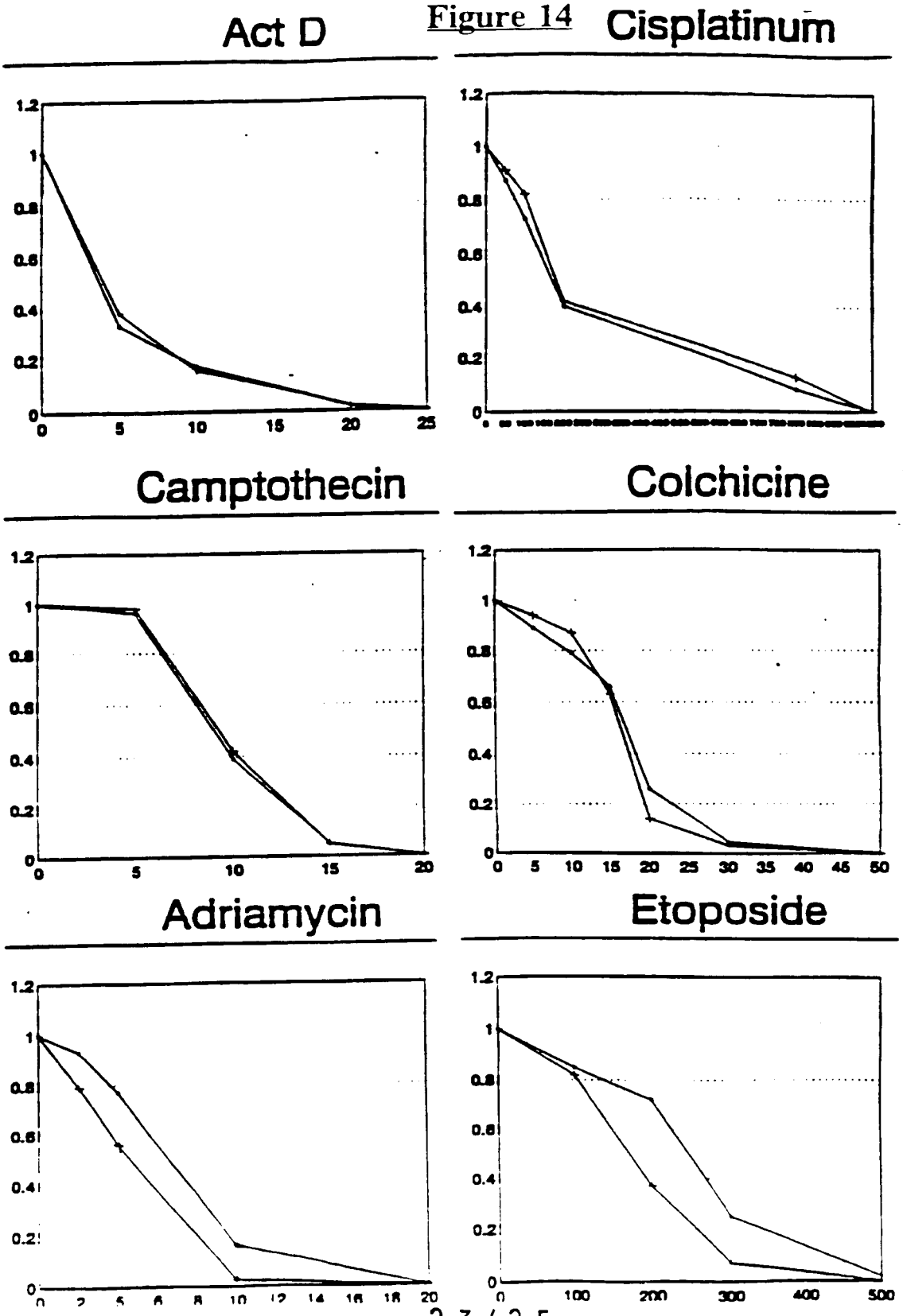
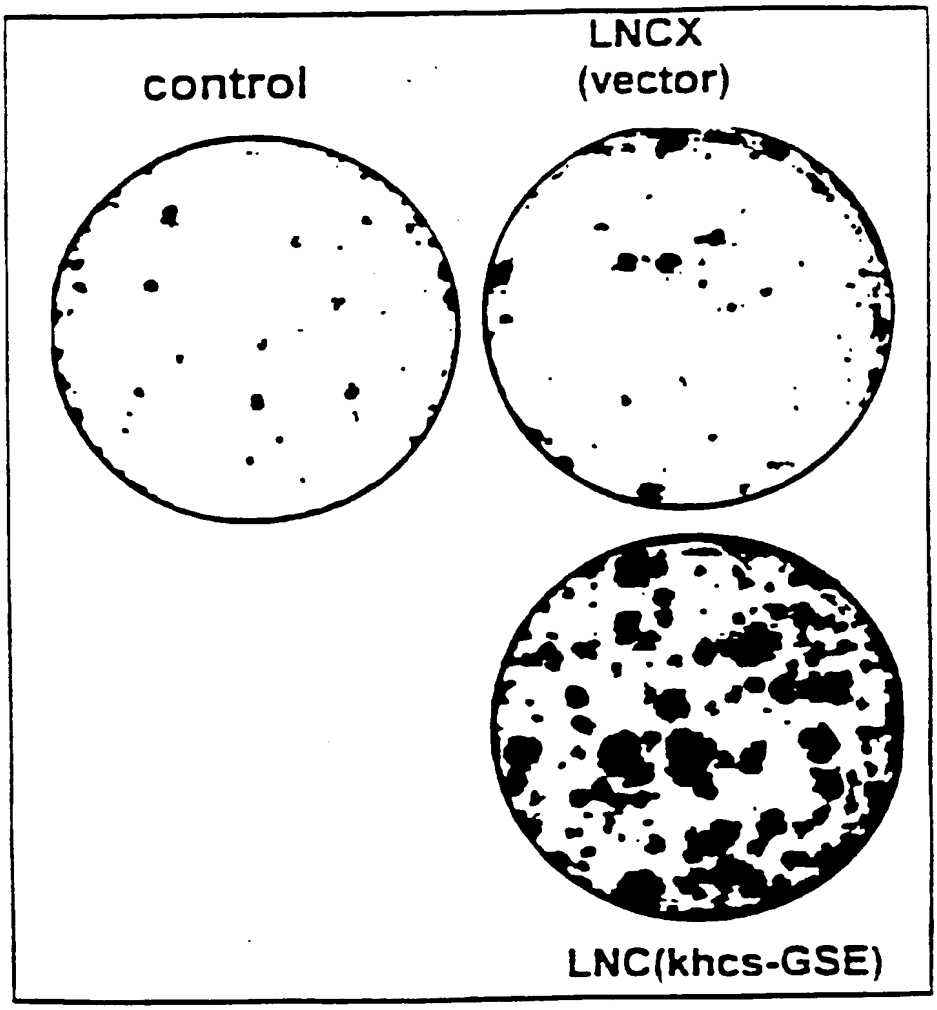
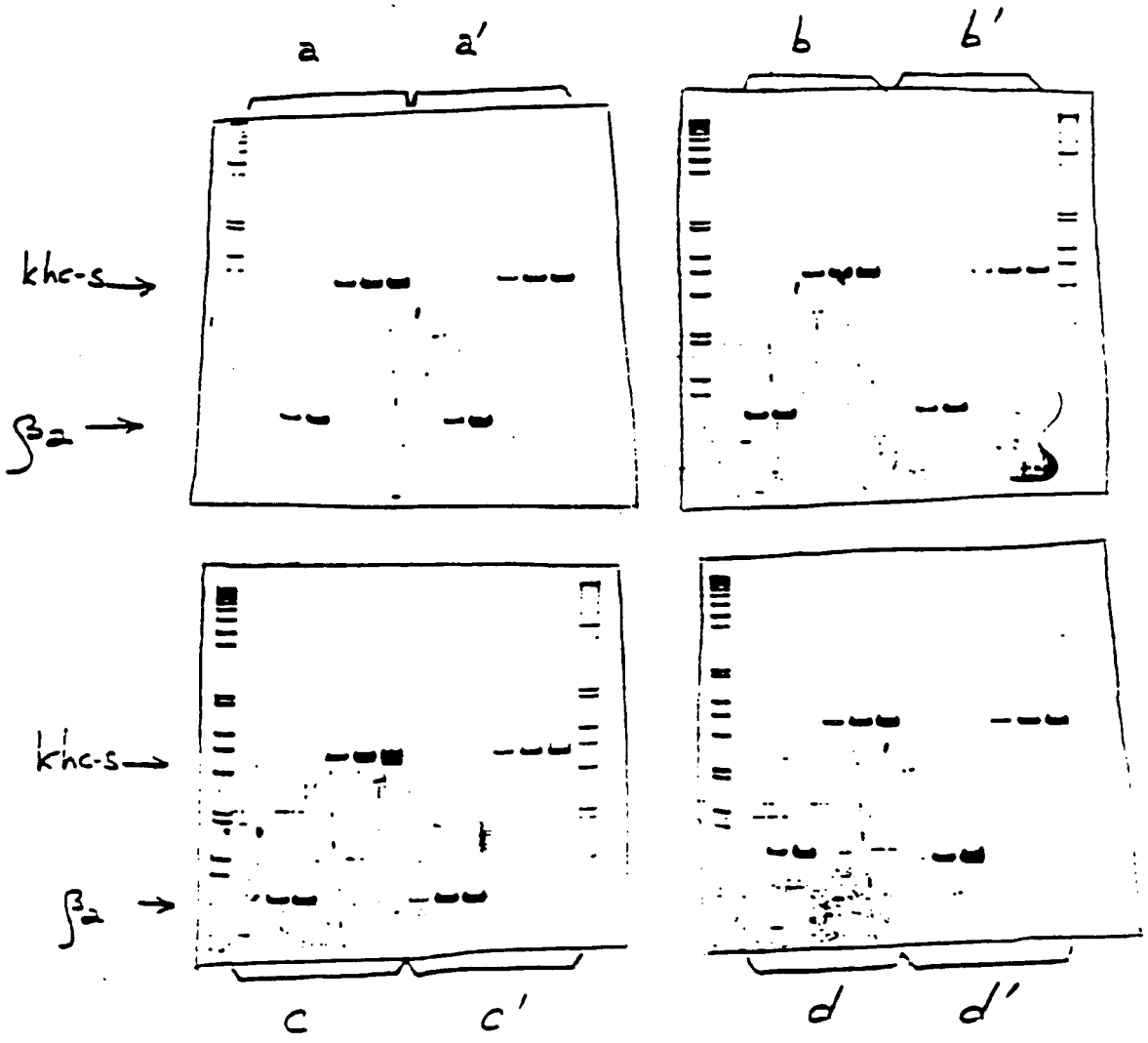

Act D
Cisplatinum
Camptothecin
Colchicine
Adriamycin
Etoposide

Figure 15



Mouse embryo fibroblasts

Figure 16



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 94/02519

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12N15/12 C12N15/67 C12N9/90 C12N15/10 C12N5/10
C12P21/08 C07K7/06 C12Q1/68 G01N33/68 A61K37/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C12N C07K C12Q G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,92 07071 (THE BOARD OF TRUSTEES OF THE UNIVERSITY OF ILLINOIS, US) 30 April 1992 cited in the application	1-33, 52-54, 61-63, 73,77, 81,85, 89,93
Y	see the whole document	34-36, 40-42, 46-48, 55-57, 64-66
	& US-A-5217889	

	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

5

Date of the actual completion of the international search	Date of mailing of the international search report
12 July 1994	14. 07. 94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Nauche, S

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JOURNAL OF BIOLOGICAL CHEMISTRY. vol. 265, no. 6 , 25 February 1990 , BALTIMORE US pages 3278 - 3283 KOSIK, K.ET AL.; 'The primary structure and analysis of the squid kinesin heavy chain.' see the whole document	34-36, 40-42, 46-48, 55-57, 64-66
X	NUCLEIC ACIDS RESEARCH. vol. 20, no. 4 , 25 February 1992 , ARLINGTON, VIRGINIA US pages 711 - 717 HOLZMAYER TA;PESTOV DG;RONINSON IB; 'Isolation of dominant negative mutants and inhibitory antisense RNA sequences by expression selection of random DNA fragments.' cited in the application	1-33, 52-54, 61-63, 67-69, 73,77, 81,85, 89,93
Y	see the whole document cited in the application	34-36, 40-42, 46-48, 55-57, 64-66
P,X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 90, no. 8 , 15 April 1993 , WASHINGTON US pages 3231 - 3235 GUDKOV AV;ZELNICK CR;KAZAROV AR;THIMMAPAYA R;SUTTLE DP;BECK WT;RONINSON IB; 'Isolation of genetic suppressor elements, inducing resistance to topoisomerase II-interactive cytotoxic drugs, from human topoisomerase II cDNA.' cited in the application see the whole document	1-33, 52-54, 61-63, 67-69, 73,77, 81,85, 89,93

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/02519

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark : although claims 67-72, as far as used in vivo, are directed to a method of treatment of the human/animal body as well as diagnostic methods (Rule 39.1(iv)PCT) the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/US 94/02519

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9207071	30-04-92	US-A-	5217889	08-06-93
		AU-B-	649759	02-06-94
		AU-A-	9025091	20-05-92
		CA-A-	2094144	20-04-92
		EP-A-	0555370	18-08-93
		JP-T-	6502309	17-03-94